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(71) Applicant *(for all designated States except US)*: SYNPHAR LABORATORIES INC. [CA/CA]; 4290–91A Street, Edmonton, Alberta T6E 5VS (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: SINGH, Rajeshwar [CA/CA]; SynPhar Laboratories Inc., 4290–91A Street, Edmonton, Alberta T6E 5VS (CA). ZHOU, Nian [CA/CA]; SynPhar Laboratories Inc., 4290–91A Street, Edmonton, Alberta T6E 5V2 (CA). REDDY, Andhe, Venkat, Narender [CA/CA]; SynPhar Laboratories Inc., 4290–91A Street, Edmonton, Alberta T6E 5V2 (CA). MICETICH, Ronald, George [CA/CA]; SynPhar Laboratories Inc., 4290–91A Street, Edmonton, Alberta T6E 5V2 (CA).

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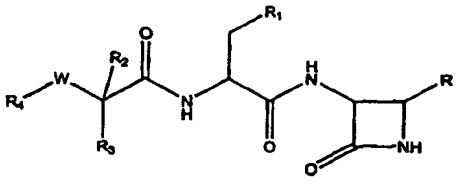
(54) Title: MONOBACTAM ENZYME INHIBITORS



(I)

(57) Abstract

Monobactam compounds of formula (I) wherein R, R_1, R_2, R_3, R_4 and W are as defined in the specification are inhibitors of cysteine proteases such as cathepsins B, L, K and S.

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Monobactam Enzym Inhibitors

This invention relates to 3-(2-disubstituted acetamido) monobactam derivatives which are inhibitors of cysteine proteases, to the use of these compounds, and to pharmaceutical compositions comprising them.

Background to the invention

Cysteine proteinases containing a highly reactive cysteine residue with free thiol group at the active site are known to play an important role in the aberrant protein turnover diseases states such as muscular dystrophy (Am. J .Pathol. 1986, 122, 193-198, Am. J. Pathol. 1987, 127, 461-466), bone resorption (Biochem. J. 1991, 279, 167-274), myocardial infarction (J. Am. Coll. Cardiol. 1983,2, 681-688), cancer metastasis (Cancer Metastasis Rev. 1990, 9, 333-352), arthritis (Biochem. Pharmacol. 1992, 44, 1201-1207; Arthritis Rheum. 1994, 37, 237-247) and pulmonary emphysema (Am. Rev. Respir. Dis. 1975,111, 579-586). A variety of cysteine proteinase have been shown to be present in mammalian tissue. The most notable of these proteinases are the lysosomal cathepsins (cathepsin B, H, K, S, and L) and the cytoplasmic Ca^{2+} dependent enzymes, the calpains. These enzymes are, therefore, excellent targets for the development of specific inhibitors as possible therapeutic agents.

The known cathepsins are synthesized on membrane bound ribosomes, transferred to the endoplasmic reticulum, then to the Golgi apparatus and finally to the lysosome and endosomes. They have an important function in regulation of intracellular protein metabolism, mobilisation of tissue proteins and conversion of proenzymes, prohormones and neuropeptides into biologically active molecules. The cathepsins are believed to be involved in a number of diseases.

Cathepsin K can be secreted into the extracellular space and is involved in bone and cartilage remodelling. Cathepsin K is implicated in the pathogenesis of osteoporosis.

Cathepsin K inhibitors can prevent osteoporosis in animal models (PNAS.1997. 94:14249-14254). Cathepsin L inhibitors have also been shown to inhibit osteoporosis (Bone, 1997. 20:465-471).

Cathepsin B and others have also been shown to be released extracellularly by various tumour cells and are thought to play a role in tumour invasion (Journal of cellular Physiology. 1992. 150:534-544).

The cathepsins have also been shown to play a role in rheumatoid arthritis (Arthritis and Rheumatism 1994. 37:236-247) and neuronal and cardiac ischaemia (European Journal of Neuroscience. 1998. 10.1723-1733).

Cathepsins S and L both play a role in the generation of free MHC class II molecules capable of binding antigenic peptides in the endosomes. These class II/peptide complexes move to the cell membrane and are involved in T lymphocyte activation. Inhibitors of Cathepsin S have been shown to inhibit allergic immune responses (Journal of Clinical Investigation. 1998. 101:2351-2363).

In addition to their role in the above diseases, cathepsins play a major role in the pathogenesis of infectious diseases. For example, cathepsins are used by the protozoal parasites Plasmodium (malaria) and Trypanosoma (Chagas Disease) to invade the human host and cathepsin inhibitors can inhibit experimental disease in both cases (Antimicrobial agents and chemotherapy. 1998. 42:2254-2258; Journal of Experimental Medicine. 1998. 188:725-734). Cathepsins are also virulence factors for several pathogenic bacteria.

A recent review (Annu. Rev. Physiol. 1997. 59:63-88) describes the state of the art of cysteine proteases, including the cathepsins, and their presumed biological functions. Another review (Exp. Opin. Ther. Patents,1998, 8(6), pp645-672) deals with cathepsin B

inhibitors as potential anti-metastatic agents.

Cysteine proteinases are inhibited by several types of peptide derived inhibitors such as peptidyl aldehyde (Eur. J. Biochem. 1982, 129, 33-41), chloromethyl ketone (Acta. Biol. Med. Ger. 1981, 40, 1503-1511), diazomethyl ketone (Biochemistry 1977,16, 5857-5861), monofluoromethyl ketone (Biochemical Pharmacology 1992 44, 1201-1207), acyloxy methyl ketone (J. Med. Chem. 1994, 37, 1833-1840), O-acyl hydroxamates (Biochem. Biophy. Research Communications 1988, 155, 1201-1206), methyl sulphonium salts (J. Biol. Chem. 1988, 263, 2768-2772) and epoxy succinyl derivatives (Agric. Biol. Chem. 1978, 42, 523-527) without significantly inhibiting other classes of proteinases.

These inhibitors, in general, have a natural peptidyl affinity group, and group reactive towards the thiol group of cysteine residues of cysteine proteinases. Natural peptidyl affinity groups are an attractive starting point for drug discovery because they are essential to virtually every biochemical process. Unfortunately, the in vivo effectiveness of such compounds is not as much as expected on the basis of in vitro inhibitory activity, possibly due to the competing activity with other proteinases and poor pharmacokinetics. Therefore, there exists a continuing need to develop new cysteine proteinase inhibitors with high selectivity and lower toxicity.

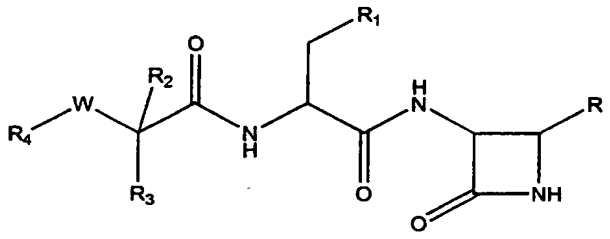
Our laboratory has been actively involved in search of novel type of cysteine proteinase inhibitors with high selectivity amongs cysteine proteinase class of enzymes. We have found that a novel class of compounds having a natural peptidyl group at C-3 of reactive group 3-amino-4-substituted azetidin-2-one, exhibit cysteine proteinase inhibitory activity and selectivity amongs cysteine proteinases, which is reported in WO 96/32408. Note that the azetidin-2-one structure is also known as, and referred to herein as "monobactam".

Summary of the invention

The present invention is based on the discovery of novel 3-(2-disubstituted acetamido)monobactam derivatives which exhibit cysteine proteinase inhibitory activity which can be used for treatment of diseases susceptible to such inhibition, such as muscular dystrophy, arthritis, bone resorption, myocardial infarction and or cancer metastasis.

Detailed Description of the Invention

In accordance to the present invention, there is provided a 3-(2-disubstituted acetamido)monobactam compound of formula (I), or a pharmaceutically acceptable salt thereof:



(I)

wherein

W is a divalent cycloalkyl radical, a bond, or a divalent $-(CH_2)_n-$ radical wherein n is an integer from 1 to 8 inclusive;

R is a group $-OCOR_5$ or a group $-X_1R_7$ wherein

R_5 is

(i) a C_1-C_6 alkyl group which may be substituted by 1 or 2 substitutents selected from

hydroxy,

halogen,
amino,
carboxy
guanidino,
amidino,
phenyl, or
phenyl substituted by 1 to 3 substituents selected from
hydroxy,
methylendioxy,
halogen,
carboxy
C_1-C_4 alkyl,
C_1-C_4 alkoxy,
cyano,
guanidino,
amidino,
amino or
-NHCOR$_6$ wherein R$_6$ is C_1-C_4 alkyl; or

(ii) an aryl group which may be substituted by 1 to 3 substituents selected from
hydroxy,
methylendioxy,
halogen,
carboxy,
C_1-C_4 alkyl,
C_1-C_4 alkoxy,
cyano,
guanidino,

amidino,
amino or
$NHCOR_6$ wherein R_6 is C_1-C_4 alkyl; or

X_1 is -O-, -S-, -S(O)-, or -$S(O_2)$-;

R_7 is

(i) a C_1-C_6 alkyl group which may be substituted by 1 to 2 substitutents selected from

hydroxy,
carboxy,
halogen,
amino,
phenyl, or
phenyl substituted by 1 to 3 substituents selected from

hydroxy,
methylendioxy,
halogen,
carboxy,
phenyl,
C_1-C_4 alkoxy,
cyano,
heterocyclyl,
C_1-C_4 alkyl, or
C_1-C_4 alkyl substituted with carboxy and/or amino;

(ii) a cycloalkyl group;

(iii) an aryl group which may be substituted by 1 to 3 substituents selected

from

hydroxy,
methylendioxy,
amino,
cyano,
halogen,
carboxy,
phenyl,
C_1-C_4 alkoxy,
C_1-C_3-haloalkyl,
heterocyclyl,
C_1-C_4 alkoxy,
cyano.
heterocyclyl,
C_1-C_4 alkyl, or
C_1-C_4 alkyl substituted with carboxy and/or amino; or

(iv) a heterocyclic group, which may be substituted by 1 or 2 substituents, selected from

hydroxy,
halogen,
carboxy,
C_1-C_4 alkyl,
C_1-C_4 alkoxy or
cyano;

R_1 is (a) an aryl group which may be substituted by 1 to 3 substitutents selected from C_1-C_4 alkyl, C_1-C_3-haloalkyl, halogen, -$NHCOR_8$, -OR_8, -NHR_8, -$N(R_8)_2$, -SR_8, phenyl, amidino, or guanidino, wherein R_8 is hydrogen, C_1-C_6 alkyl, C_3-C_6

cycloalkyl or phenyl; or

(b) a heterocyclic group, which may be substituted by 1 to 2 substitutents, selected from C_1-C_4 alkyl, C_1-C_4 alkoxy, hydroxy, halogen, carboxy, amino, cyano or phenyl; or

(c) cycloalkyl; or

(d) C_1-C_4 alkyl;

R_2 is hydrogen and R_3 is hydrogen or an amino or mono- or di-(C_1-C_6)alkylamino group, an acylamino group, or an aryloxycarbonyl- or (C_1-C_6)alkoxycarbonylamino group; or

R_2 and R_3 taken together with the carbon atom to which they are attached form a cycloalkyl ring; or

R_2 and R_3 taken together represent a group =NOR_9 wherein R_9 is (C_1-C_6)alkyl optionally substituted by an amino, aryloxycarbonyl- or (C_1-C_6)alkoxycarbonylamino group; and

R_4 is (a) a group -NH-C(=NR_{11})R_{10} wherein R_{10} is amino, mono- or di-(C_1-C_6)alkylamino, protected amino, or (C_1-C_6)alkyl, and R_{11} is hydrogen, (C_1-C_6)alkyl, or an N-protecting group; or

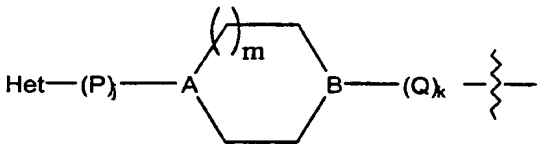
(b) a (C_2-C_{12})alkyl-, cycloalkyl(C_2-C_{12})alkyl-, heterocyclyl(C_2-C_{12})alkyl-, aryl(C_2-C_{12})alkyl-, heteroaryl(C_2-C_{12})alkyl- group, the (C_2-C_{12})alkyl part of which which is interrupted by one or more non-adjacent O or S atoms; or

(c) a (C_1-C_6)alkoxy-, aryloxy-, cycloalkyl(C_1-C_6)alkoxy-, heterocyclyl(C_1-C_6)alkoxy-, aryl(C_1-C_6)alkoxy-, heteroaryl(C_1-C_6)alkoxy-, (C_1-C_6)alkylthio-, arylthio-,

cycloalkyl($(C_1$-$C_6)$alkylthio-, heterocyclyl$(C_1$-$C_6)$alkylthio-, aryl$(C_1$-$C_6)$alkylthio-, or heteroaryl$(C_1$-$C_6)$alkylthio- group any of which may be substituted by hydroxy, methylenedioxy, $(C_1$-$C_4)$alkoxy, $(C_1$-$C_4)$alkyl, halogen, cyano, carboxy, amino, or a group -NH-C(=NH)R_{12} wherein R_{12} is amino or $(C_1$-$C_6)$alkyl; or

(d) an aryl, cycloalkyl, or heterocyclic group, or an amino group substituted with an N-containing heterocyclic group; or

(e) a group of formula F



(F)

wherein P and Q are independently -C(=O)-, -S(O_2)- or -CH_2-, provided at least one of P and Q is not -CH_2-; j, k and m are independently 0 or 1, provided at least one of j and k is 1; A and B are independently nitrogen or carbon atoms, provided at least one of A and B is a nitrogen atom; and "Het" is a heterocyclic group having at least one ring nitrogen atom;

(f) a group -NHCO$CH_2O$$R_{13}$, -NHCO$CH_2S$$R_{13}$, -NHCO$CH_2SO_2$$R_{13}$, or -COO$R_{13}$ wherein R_{13} is $(C_1$-$C_6)$alkyl, aryl, heterocyclic or cycloalkyl, which may be substituted by 1-3 substituents selected from hydroxy, methylenedioxy, halogen, cyano, carboxy, guanidino, amidino, $(C_1$-$C_4)$alkyl, $(C_1$-$C_4)$alkoxy, amino or phenyl.

Pharmaceutically acceptable salts of the compounds of this invention include the sodium, potassium, magnesium, calcium, hydrogen chloride, tartaric acid, succinic acid,

fumaric acid and p-toluenesulfonic acid salts.

As used herein the term "(C_1-C_6)alkyl" or "lower alkyl" means a straight or branched chain alkyl moiety having from 1 to 6 carbon atoms, including for example, methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 2-methylprop-1-yl, 2-methylprop-2-yl, pentyl, 3-methylbutyl, and hexyl.

The term "cycloalkyl" means a saturated alicyclic moiety having from 3-7 carbon atoms and includes, for example, cyclohexyl, cycloheptyl, cyclopentyl, cyclobutyl and cyclopropyl.

The term "aryl" refers to a mono-, bi- or tri-cyclic, substituted or unsubstituted, carbocyclic aromatic group, and to groups consisting of two covalently linked substituted or unsubstituted monocyclic carbocyclic aromatic groups. Illustrative of such groups are phenyl, biphenyl and napthyl. Examples include C_6-C_{12} aryl groups such as phenyl, biphenyl, naphthyl, tetrahydronaphthyl, dihydronaphthyl, and cyclohexyl phenyl.

The unqualified term "heterocyclyl" or "heterocyclic" means a 5-7 membered heterocyclic ring, which may be aromatic or non-aromatic, containing one or more heteroatoms selected from S, N and O, and optionally fused to a benzene or hetero-atom containing ring. The term therefore includes C_1-C_{11} heterocyclic groups containing 1-4 heteroatoms selected from nitrogen; sulfur or oxygen. Examples include thienyl, pyridyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,3,4-tetrazolyl, imidazolyl, quinolinyl, isoquinolinyl, indolyl, pyrimidinyl, benzofuranyl, benzothienyl, morpholinyl, thiomorpholinyl, piperazinyl, piperidinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, pyridylphenyl, pyrimidylphenyl, pyrrolyl, furyl, thienyl, piperidinyl, imidazolyl, oxazolyl, thiazolyl, thiadiazolyl, pyrazolyl, pyridinyl, pyrrolidinyl, pyrimidinyl, morpholinyl, piperazinyl, indolyl, benzimidazolyl, maleimido, succinimido, phthalimido, 1,2-dimethyl-3,5-dioxo-1,2,4-triazolidin-4-yl, 3,4,4-trimethyl-2,5-dioxo-1-imidazolidinyl, 2-methyl-3,5-

dioxo-1,2,4-oxadiazol-4-yl, 3-methyl-2,4,5-trioxo-1-imidazolidinyl, 2,5-dioxo-3-phenyl-1-imidazolidinyl, 2-oxo-1-pyrrolidinyl, 2,5-dioxo-1-pyrrolidinyl or 2,6-dioxopiperidinyl, or (ii) a naphththalimido (ie 1,3-dihydro-1,3-dioxo-2H-benz[f]isoindol-2-yl), 1,3-dihydro-1-oxo-2H-benz[f]isoindol-2-yl, 1,3-dihydro-1,3-dioxo-2H-pyrrolo[3,4-b]quinolin-2-yl, or 2,3-dihydro-1,3-dioxo-1H-benz[d,e]isoquinolin-2-yl group.

Unless otherwise specified in the context in which it occurs, the term "substituted" as applied to any moiety herein means substituted with up to four substituents, each of which independently may be (C_1-C_6)alkoxy, phenoxy, hydroxy, mercapto, (C_1-C_6)alkylthio, amino, halo (including fluoro, chloro, bromo and iodo), trifluoromethyl, nitro, -COOH, -$CONH_2$, -$COOR^A$, -$NHCOR^A$, -$CONHR^A$, -NHR^A, -NR^AR^B, or -$CONR^AR^B$ wherein R^A and R^B are independently a (C_1-C_6)alkyl group.

The term "protecting group" when used in relation to an amino or carboxylic acid moeity in the compounds of this invention means a group which is used to render the amino or carboxylic acid moeity substantially non reactive, ie to neutralise its amino or carboxylic acid functionality. In this context, protected amino groups include amido and acylamino, protected hydroxy or mercapto groups include ethers and thioethers, protected carboxyl groups include esters, and imidazolyl, indolyl or guanidyl groups may be protected as t-butoxycarbonyl derivatives. These are only examples of the many protecting derivatives known in the art, and others will be known to the skilled man. Such protecting groups are of course well known, eg from the art of peptide synthesis, and are discussed in the widely used handbook by T.W. Greene and P.G.M. Wuts, Protective groups in Organic Synthesis, 2nd Edition, Wiley, New York 1991, and elsewhere in the chemical literature.

In the compounds of the invention, where R_1 is aryl or heterocyclic substituted by C_1-C_6 haloalkyl, the haloalkyl group may be fluoromethyl, fluoroethyl, fluoropropyl, chloroethyl, chloropropyl, bromoethyl, bromopropyl, 1-fluoromethyl ethyl and the like.

The azetidinone nucleus carries two asymmetric carbon atoms at position 3 and 4, and can exist as 4- diastereoisomers. In general, the preferred isomer is that in which the hydrogen atoms at C3 and C4 are cis to each other for superior inhibitory activity against different cysteine proteinase such as papain, Cathepsin B, Cathepsin H, Cathepsin K, Cathepsin S and Cathepsin L. Such diasterioisomers and their racemates are also included within use of the azetidinone derivatives as cysteine proteinase inhibitor.

A particular class of 3-(2-disubstituted acetamido)monobactam derivatives of the invention has general formula I above, wherein

R is selected from acetoxy, butyloxy, 2-carboxy ethyloxy, 2-aminoethyloxy, 2-fluoro ethoxy, cyclopentyloxy, cyclohexyloxy, cyclohexylthio, phenoxy, methyl phenoxy, naphthyloxy, morpholino phenyloxy, 2-hydroxy ethylthio, phenylthio, phenylsulphonyl, 4-(2-carboxy-2-amino ethyl)-phenoxy, 4-carboxy phenoxy, 3-carboxy phenoxy, 2-pyridylthio, 4-pyridylthio, benzyloxy, 3-pyridyl phenoxy, 3-tetrazolyl phenoxy, 3,4-methylenedioxy phenoxy, 3, 4-ethylenedioxy phenoxy, hydroxy phenoxy, amino phenoxy, acetamido phenoxy, trifluoromethyl phenoxy, t-butyl phenoxy, cyano phenoxy, tetrahydroquinolinoxy, quinolinoxy, quinolinthio, and the like.

R_1 is selected from phenyl, pyridyl, naphthyl, biphenyl, cyclohexyl, thienyl, halophenyl, hydroxy phenyl, methoxyphenyl, dimethoxy phenyl, 3,4-methylenedioxy phenyl, 3,4-ethylenedioxy phenyl, triflouromethyl phenyl, benzothienyl, thiazolyl, 3-iodo-4-hydroxyphenyl, quinolinyl, isoquinolinyl, tetrahydroquinolinyl, tetrahydronaphthyl, aminonaphthyl, acetamidonaphthyl, isopropyl phenyl, t-butylphenyl, and the like.

W is a divalent cyclopropyl, cyclopentyl or cyclohexyl radical, a bond, or a

divalent $-(CH_2)_n-$ radical wherein n is 1, 2, 3, 4, 5, 6, 7, or 8.

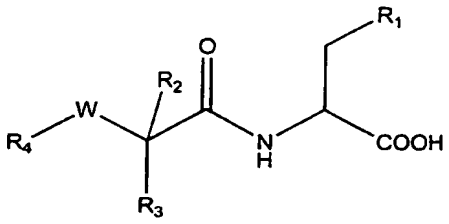
R_2 and R_3 independently are hydrogen, methyl, phenyl, hydroxy, amino, benzyloxycabonyl amino, t-butyloxycarbonyl amino, acetamido and the like, or R_2 and R_3 together are aminoethoxyimino, guanidinoethoxyimino, amidinoethoxyimino, imdazolylethoxyimino, pyridylethoxyimino and the like.

R_4 is selected from guanidino, thienyl, dibenzyloxycarbonylguanidino, di-t-butyloxycarbonylguanidino, methoxyethoxy, (methoxyethoxy)ethoxy, acetimidoylamino, t-butylamino, amidinophenylamino, guanidinophenylamino, amidinophenylaminocarbonyl, guanidinophenylaminocarbonyl, amidinophenoxy, guanidinophenoxy, cyclohexyloxy, phenoxy, naphthyloxy, benzyloxycarbonylamino, t-butyloxycarbonylamino, cyclohexylamino, cyclohexylaminocarbonyl, biotinylamino, carboxymethoxyacetamido, pyridylthioacetamido, carboxymethylthioacetamido, benzotriazoloxy, triazolylamino, pyridylamino, pyrimidylamino, imidazolylamino, pyrimidylpiperazinyl, pyridylpiperazinyl, phenylpiperazinyl, quinazolinyloxy, quinolinylamino, quinolinyloxy, dimethoxyphenoxy, cyanophenoxy, N-carbonyl piperazine, N, N1-dicarbonyl piperazine, N-carbonyl-N1-sulphonyl piperazine, 4-carbonyl piperidine, N-carbonyl piperadine, N-sulphonyl piperadine, 3-carbonyl pyrrolidine, N-carbonyl pyrrolidine, N-sulphonyl pyrrolidine, N-carbonyl alkyl-N1-sulphnoyl piperazine, N-sulphonyl alkyl-N1-carbonyl piperazine and the like. and the like.

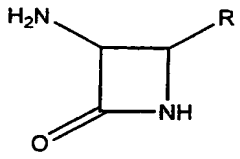
Specific Examples of compounds of the invention include those disclosed in the Examples herein, and diasteroisomers thereof.

Compounds of the invention may be prepared by, or by analogy with, the preparative methods described in the Examples herein. Thus compounds may be prepared by

condensation of a monobactam of formula (III) with a carboxylic acid of formula (II):



(II)

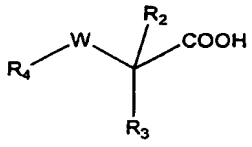


(III)

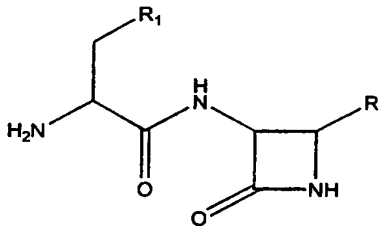
The condensation of (II) and (III) may be carried out using dicyclohexylcarbodiimide (DCC), or by using an activated ester or acid chloride of (II), according to methods known in the art.

Compounds III wherein R is $OCOR_5$ may be prepared by the synthetic route described in Eur. J. Med. Chem 1992, 27, 131-140, and Tetrahedron 1983, 39, 2577-2589.

Alternatively, compounds of the invention may be prepared by condensation of a compound (IV) with a compound (V)



(IV)



(V)

This condensation may also be carried out using dicyclohexylcarbodiimide (DCC), or by using an activated ester or acid chloride of (IV), according to methods known in the art.

Compounds (V) may be prepared by reacting compound III with the appropriate N-protected-2-substituted alanine, and the N-protecting group, for example benzyloxycarbonyl or t-butoxycarbonyl, may be removed and the free amine (V) coupled to (IV) as described.

3-(2-Disubstituted acetamido)monobactam derivatives of general formula I wherein R is $-XR_7$ may prepared by starting from compound of general formula I wherein R is $OCOCH_3$ by reacting with R_7XH in presence of lewis acids such as zinc acetate, zinc iodide, zinc chloride, titanium tetrachloride, palladium acetate, boron trifluoride, aluminium trichloride and the like or in presence of base such as sodium hydroxide. In cases where a carboxy group is substituent in R_7, it may be protected with diphenyl methyl or 1,1-dimethyl ethyl, and where an amino group is a substituent in R_7 it may be protected with benzyloxy carbonyl or 1,1-dimethyl ethoxy carbonyl, and the protecting groups subsequently removed.

3-(2-Disubstituted acetamido)monobactam derivatives of general formula I wherein R is $-SR_7$ may be converted to, $-SOR_7$, or $-SO_2R_7$ by oxidation with oxidizing agent selected from m-chloroperbenzoic acid, hydrogen peroxide. peracetic acid, potassium permagnet, magnese dioxide and the like.

In the above reactions, the reactants may be reacted together in solvent at elevated or low temperatures for sufficient time to allow the reaction to proceed essentially to completion. The reaction conditions will depend upon the nature and reactivity of the reactants. Wherever a base is used in a reaction, it may be selected from triethylamine, pyridine, 4-dimethylaminopyridine, diisopropylethylamine, 1,5-diazabicyclo[4,3,0]non-5-ene, 1,8-diazabicyclo[5,4,0]undec-7-ene, sodium carbonate, potassium carbonate or cesium carbonate.

The solvents of choice for the reaction may be selected from non reactive solvents,

depending on the reactants, such as benzene, toluene, acetonitrile, tetrahydrofuran, ethanol, methanol, chloroform, ethyl acetate, methylene chloride, dimethyl formamide, dimethyl sulfoxide, hexamethyl phosphoric triamide, or the like. Solvent mixtures may also be utilized.

Reaction temperatures would generally range from between -70°C to 150°C. The preferred molar ratio of reactants are 1:1 to 5.0. The reaction time may range from 0.5 to 72 hours, depending on the reactants.

The deprotection of N-protective group is carried out either by hydrogenation or by hydrolysis with appropiate acids such as hydrochloric acid, trifluoroacetic acid or acetic acid in solvent such as methanol, ethanol, propanol or ethyl acetate. The hydrogenation reaction usually carried out in the presence of a metal catalyst, such as Pd, Pt, or Rh, under normal pressure to high pressure.

The compounds of this invention, when used alone or in combination with other drugs as an agent for treating muscular dystrophy, arthritis, osteoporosis or cancer metastasis in mammals including humans, may be in pharmaceutical dosage forms including parenteral preparation such as injections, suppositories, aerosols and the like, and oral preparations such as tablets, coated tablets, powders, granules, capsules, liquids and the like. Injections are generally preferred. The above preparatins are formulated in a manner known in the art.

For the formulation of solid preparations for oral administration, an excipient, and if desired, a binder, disintegrator, lubricant, coloring agent, corrigent, flavor etc. are added to the compound of the invention, and then tablets, coated tablets, granules, powders, capsules or the like are prepared in a conventional manner.

For the formulation of injections, a pH adjusting agent, buffer, stabilizer, isotonic agent,

local anesthetic or the like is added to the active ingredient of the invention, and injections for subcutaneous, intramuscular or intravenous administration can be prepared in the conventional manner.

For the formulation of suppositories, a base, and if desired, a surfactant are added to the active ingredient of the invention, and the suppositories are prepared in a conventional manner.

The excipients useful for solid preparations for oral administration are those generally used in the art, and the useful examples are excipients such as lactose, sucrose, sodium chloride, starches, calcium carbonate, kaolin, crystalline cellulose, methyl cellulose, glycerin, sodium alginate, gum arabic and the like, binders such as polyvinyl alcohol, polyvinyl ether, polyvinyl pyrrolidone, ethyl cellulose, gum arabic, schellac, sucrose, water, ethanol, propanol, carboxymethyl cellulose, potassium phosphate and the like, lubricants such as magnesium stearate, talc and the like, and further include additives such as usual known coloring agents, disintegrators and the like. Examples of bases useful for the formulation of suppositories are oleaginous bases such as cacao butter, polyethylene glycol, lanolin, fatty acid triglycerides, witepsol (trademark, Dynamite Nobel Co. Ltd.) and the like. Liquid preparations may be in the form of aqueous or oleaginous suspension, solution, syrup, elixir and the like, which can be prepared by a conventional way using additives.

The amount of the compound I of the invention to be incorporated into the pharmaceutical composition of the invention varies with the dosage form, solubility and chemical properties of the compound, administration route, administration scheme and the like. The amount may be about 1 to 25 w/w% in the case of oral preparations, and about 0.1 to about 5 w/w% in the case of injections which are parenteral preparations.

The dosage of the compound I of the invention is suitably determined depending on the

individual cases taking symptoms, age and sex of the subject and the like into consideration. Usually the dosage in the case of oral administration is about 50 to 1500 mg per day for an adult in 2 to 4 divided doses, and the dosage in the case of injection, for example, by intravenous administration is 2 ml (about 1 to 100 mg) which is administered once a day for adults wherein the injection may be diluted with physiological saline or glucose injection liquid if so desired, and slowly administered over at least 5 minutes. The dosage in case of suppositories is about 1 to 1000 mg which is administered once or twice a day at an interval of 6 to 12 hours wherein the suppositories are administered by insertion into the rectum.

The following Examples illustrate embodiments of the invention.

Example 1

(3S, 4S)-3-[2S-2-(4-guanidinobutanoyl)-amino-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one hydrochloride salt

(3S, 4S)-3-benzyloxycarbonylamino-4-phenoxy-azetidin-2-one (2.08 g, 6.68 mmole) is hydrogenated with 1.5 g of 10 % palladium on activated carbon in ethyl acetate (60 ml) at 50 psi hydrogen pressure at room temperature for 2 hrs. After removal of catalyst by filtration, deprotected (3S, 4S)-3-amino-4-phenoxy-azetidin-2-one in ethyl acetate is obtained.

To a solution of t-butyloxycarbonyl-L-phenylalanine (1.77 g, 6.68 mmole) and 1-hydroxybenzotriazole (902 mg, 6.68 mmole) in THF (30 ml), DCC (1.37 g, 6.68 mmole) is added at 0 °C. The reaction mixture is stirred at 0 °C for 30 min and room temperature for 1 hr and then cooled with an ice bath. The resulting DCU is removed by filtration. Then a pre-cooled solution of (3S, 4S)-3-amino-4-phenoxy-azetidin-2-one in ethyl acetate is added at 0 °C and the resulting mixture is stirred at 0 °C for 1 hr and room temperature for 1 hr. After removal of solvent, the residue is dissolved in ethyl

acetate, washed with saturated $NaHCO_3$ solution, water, brine and dried over sodium sulfate. After removal of solvent, the residue is dissolved in THF (20 ml) and cooled with an ice bath and the resulting DCU is removed by filtration. After removal of solvent, the solid is washed with ether and 2.4 g of (3S, 4S)-3-[2S-2-(t-butyloxycarbonylamino)-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one.

To a solution of (3S, 4S)-3-[2S-2-(t-butyloxycarbonylamino)-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one (2.6 g, 6.1 mmole) and anisole (0.5 ml) in dichloromethane (5 ml), trifluoroacetic acid (10 ml) is added at 0 °C. The reaction mixture is stirred at 0 °C for 1 hr. at room temperature for 30 min. The solution is evaporated to dryness in vacuo and the residue triturated with ether. After removal of solvent, the residue is dissolved in ethyl acetate and washed with cold saturated $NaHCO_3$ solution, water, brine and dried over sodium sulfate. After removal of solvent, the residue is purified by silica gel column chromatography using methanol-ethyl acetate as eluent and 1.2 g of (3S, 4S)-3-(2S-2-amino-2-benzyl-acetamido)-4-phenoxy-azetidin-2-one is obtained.

To a solution of 1-(N, N'-dibenzyloxycarbonyl-amidino)-pyrazole (1.512 g, 4 mmole) (ref: Tetrahedron Letter, 1993, 34, 3389-3392) and 4-aminobutanoic acid (412 mg, 4 mmole) in DMF (20 ml), triethylamine (0.84 ml, 6 mmole) and H_2O (10 ml) are added at 0 °C. The mixture is stirred at 0 °C for 2 hrs and at room temperature overnight. The reaction mixture is diluted with ethyl acetate/ether and cold water and the pH is adjusted to pH 2, washed with water, brine and dried over sodium sulfate. After removal of solvent, the solid is washed with ether to give 1.4 g of 4-(N,N'-dibenzyloxycarbonyl-guanidino)-butanoic acid as white solid.

To a solution of 4-(N,N'-dibenzyloxycarbonyl-guanidino)-butanoic acid (820 mg, 2 mmole), (3S, 4S)-3-(2S-2-amino-2-benzyl-acetamido)-4-phenoxy-azetidin-2-one (552 mg, 1.7 mmole) and BOP (884 mg, 2 mmole) in DMF (10 ml), triethylamine (0.28 ml, 2 mmole) is added at 0 °C. The reaction mixture is stirred at room temperature overnight and then diluted with ethyl acetate (200 ml) and ether (100 ml), washed with saturated $NaHCO_3$ solution, water, brine and dried over sodium sulfate. After removal of solvent, the residue is purified by silica gel column chromatography using hexane-ethyl acetate

as eluent to give 1.1 g of (3S, 4S)-3-{2S-2-[4-(N,N'-dibenzyloxycarbonyl-guanidino)-butanoyl] amino-2-benzyl-acetamido}-4-phenoxy-azetidin-2-one is obtained.

(3S, 4S)-3-{2S-2-[4-(N,N'-dibenzyloxycarbonyl-guanidino)-butanoyl] amino-2-benzyl-acetamido}-4-phenoxy-azetidin-2-one (1.3 g, 1.8 mmole) is hydrogenated with 1.3 g of 10 % palladium on activated carbon in ethyl acetate (50 ml)/THF (40 ml) and 1N HCl (2.16 ml, 2.16 mmole) at 50 psi hydrogen pressure at room temperature for 2 hrs. The solid is filtered, washed with ethyl acetate and then extracted with a mixture of water/acetonitrile (3:7) (100 ml). After removal of solvent under vacuum and lyophilization, 720 mg of the title compound is obtained as white solid.
Yield: 82 %; m.p.: 118.5-120 °C
^{1}H-NMR (DMSO-d_6), δ (ppm): 1.50-1.70 (2H, m), 2.05-2.20 (2H, m), 2.70-3.15 (4H, m), 4.45-4.60 (1H, m), 4.63 (1H, d, J=8.1 Hz), 5.52 (1H, s), 6.86 (2H, d, J=8.0 Hz), 7.0-7.4 (12H, m), 7.45-7.60 (1H, br), 8.27 (1H, d, J=8.4 Hz), 8.90 (1H, d, J=8.1 Hz), 9.33 (1H, s).

Example 2

(3S, 4S)-3-[2S-2-(4-guanidinobutanoyl)-amino-2-(naphth-2-yl)methyl-acetamido]-4-phenoxy-azetidin-2-one hydrochloride salt

By a similar method as the method to synthsize (3S, 4S)-3-(2S-2-amino-2-benzyl-acetamido)-4-phenoxy-azetidin-2-one (example 1), (3S, 4S)-3-[2S-2-amino-2-(naphth-2-yl)methyl-acetamido]-4-phenoxy-azetidin-2-one is obtained from (3S, 4S)-3-amino-4-phenoxy-azetidin-2-one and t-butyloxycarbonyl-β-(2-naphthyl)-L-alanine.

To a solution of 4-(N,N'-dibenzyloxycarbonyl-guanidino)-butanoic acid (155 mg, 0.38 mmole) and 1-hydroxybenzotriazole (55 mg, 0.41 mmole) in THF (5 ml), DCC (78 mg, 0.38 mmole) is added. The reaction mixture is stirred at room temperature for 1 hr and cooled with an ice bath. the resulting DCU is removed by filtration. (3S, 4S)-3-[2S-2-amino-2-(naphth-2-yl)methyl-acetamido]-4-phenoxy-azetidin-2-one (141 mg, 0.38mmole) is added and stirred at room temperature overnight. After removal of solvent, the residue is dissolved in ethyl acetate, washed with saturated $NaHCO_3$

solution, water, brine and dried over sodium sulfate. After removal of solvent, the residue is purified by recrystallization from THF/ethyl acetate/hexane and 90 mg of (3S, 4S)-3-[2S-2-(4-N,N'-dibenzyloxycarbonylguanidino butanoyl) amino-2-(naphth-2-yl)methyl-acetamido]-4-phenoxy-azetidin-2-one is obtained.

(3S, 4S)-3-[2S-2-(4-N,N'-dibenzyloxycarbonylguanidino butanoyl) amino-2-(naphth-2-yl)methyl-acetamido]-4-phenoxy-azetidin-2-one (88 mg, 0.11 mmole) is hydrogenated with 200 mg of 10 % palladium on activated carbon in ethyl acetate (10 ml)/THF (10 ml) and 1N HCl (0.17 ml, 0.17 mmole) at 50 psi hydrogen pressure at room temperature for 2 hrs. The solid is filtered, washed with ethyl acetate and then extracted with a mixture of water/acetonitrile (3:7). After removal of solvent under vacuum and lyophilization, 40 mg of the title compound is obtained as white solid.
Yield: 65 %; m.p.: 180.5-182.5 °C
^{1}H-NMR (DMSO-d_6), δ (ppm): 1.5-1.7 (2H, m), 2.1-2.3 (2H, m), 2.8-3.3 (4H, m), 4.55-4.75 (1H, m), 4.65 (1H, d, J=8.1 Hz), 5.58 (1H, s), 6.8-7.5 (12H, m), 7.75-7.95 (5H, m), 8.43 (1H, d, J=8.1 Hz), 9.10 (1H, d, J=8.4 Hz), 9.39 (1H, s).

Example 3

(3S, 4S)-3-[2S-2-(3-guanidinopropanoyl)-amino-2-(naphth-2-yl)methyl-acetamido]-4-phenoxy-azetidin-2-one hydrochloride salt

By a similar method as desribed in example 2, the title compound is obtained from 3-(N,N'-dibenzyloxycarbonyl-guanidino)-propanoic acid and (3S, 4S)-3-[2S-2-amino-2-(naphth-2-yl)methyl-acetamido]-4-phenoxy-azetidin-2-one.
Yield: 51 %; m.p.: 153-155 °C
^{1}H-NMR (DMSO-d_6), δ (ppm): 2.35-2.45 (2H, m), 3.00-3.30 (4H, m), 4.55-4.75 (1H, m), 4.62 (1H, d, J=8.0 Hz), 5.55 (1H, s), 6.75-7.50 (13H, m), 7.70-7.90 (4H, m), 8.48 (1H, d, J=8.1 Hz), 8.98 (1H, d, J=8.0 Hz), 9.35 (1H, s).

Example 4

(3S, 4S)-3-[2S-2-(5-guanidinopentanoyl)-amino-2-(naphth-2-yl)methyl-acetamido]-4-

phenoxy-azetidin-2-one hydrochloride salt

By a similar method as desribed in example 2, the title compound is obtained from 5-(N,N'-dibenzyloxycarbonyl-guanidino)-pentanoic acid and (3S, 4S)-3-[2S-2-amino-2-(naphth-2-yl)methyl-acetamido]-4-phenoxy-azetidin-2-one.

Yield: 28 %; m.p.: 172-174 °C

^{1}H-NMR (DMSO-d_6), δ (ppm): 1.2-1.5 (4H, m), 2.0-2.2 (2H, m), 2.8-3.3 (4H, m), 4.55-4.75 (1H, m), 4.64 (1H, d, J=8 Hz), 5.55 (1H, s), 6.8-8.0 (16H, m), 8.42 (1H, d, J=8.2 Hz), 8.65 (1H, br), 9.04 (1H, d, J=8.0 Hz), 9.35 (1H, s).

Example 5

(3S, 4S)-3-[2S-2-(6-guanidinohexanoyl)-amino-2-(naphth-2-yl)methyl-acetamido]-4-phenoxy-azetidin-2-one hydrochloride salt

By a similar method as desribed in example 2, the title compound is obtained from 6-(N,N'-dibenzyloxycarbonyl-guanidino)-hexanoic acid and (3S, 4S)-3-[2S-2-amino-2-(naphth-2-yl)methyl-acetamido]-4-phenoxy-azetidin-2-one.

Yield: 19 %; m.p.: 163.7-165.8 °C

^{1}H-NMR (DMSO-d_6), δ (ppm): 1.0-1.2 (2H, m), 1.2-1.5 (4H, m), 2.0-2.15 (2H, m), 2.75-3.35 (4H, m), 4.5-4.7 (2H, m), 5.55 (1H, s), 6.8-8.1 (17H, m), 8.44 (1H, d, J=8.2 Hz), 9.0-9.3 (2H, br).

Example 6

(3S, 4S)-3-[2S-2-(6-guanidinohexanoyl)-amino-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one hydrochloride salt

By a similar method as desribed in example 1, the title compound is obtained from 6-(N,N'-dibenzyloxycarbonyl-guanidino)-hexanoic acid and (3S, 4S)-3-[2S-2-amino-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one.

Yield: 49 %

m.p.: 152.5-154.5 °C

^{1}H-NMR (DMSO-d_6), δ (ppm): 0.9-1.8 (19H, m), 2.05-2.25 (2H, m), 3.0-3.20 (2H, m),

4.25-4.45 (1H, m), 4.65 (1H, d, J=8 Hz), 5.53 (1H, s), 6.8-7.4 (9H, m), 7.5-7.65 (1H, m), 8.07 (1H, d, J=8 Hz), 8.82 (1H, d, J=8 Hz), 9.30 (1H, s).

Example 7

(3S, 4R)-3-[2S-2-(6-guanidinohexanoyl)-amino-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one hydrochloride salt

By a similar method as desribed in example 1, the title compound is obtained from 6-(N,N'-dibenzyloxycarbonyl-guanidino)-hexanoic acid and (3S, 4R)-3-[2S-2-amino-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one.

Yield: 51 %; m.p.: 162-164 °C

^{1}H-NMR (DMSO-d_6), δ (ppm): 0.8-1.7 (19H, m), 2.0-2.2 (2H, m), 3.0-3.2 (2H, m), 4.3-4.45 (1H, m), 5.30 (1H, m), 5.71 (1H, d, J=3.8 Hz), 6.85-7.40 (9H, m), 7.55-7.75 (1H, m), 7.85-7.95 (1H, m), 8.64 (1H, d, J=8 Hz), 9.26 (1H, s).

Example 8

(3S, 4S)-3-{2S-2-[2-(2-methoxyethoxy)acetamido-2-benzyl-acetamido}-4-phenoxy-azetidin-2-one hydrochloride salt

To a solution of 2-(2-methoxyethoxy)acetic acid (45 mg, 0.33 mmole) and (3S, 4S)-3-(2S-2-amino-2-benzyl-acetamido)-4-phenoxy-azetidin-2-one (98 mg, 0.3 mmole) in DMF (3 ml), BOP (146 mg, 0.33 mmole) and triethylamine (33 mg, 0.33 mmole) are added. The reaction mixture is stirred at room temperature overnight and then diluted with ethyl acetate (50 ml) and ether (50 ml), washed with saturated $NaHCO_3$ solution, water, brine and dried over sodium sulfate. After removal of solvent, the solid is washed with ether and 120 mg of the title compound is obtained.

Yield: 90 %

^{1}H-NMR (DMSO-d_6), δ (ppm): 2.85-3.20 (2H, m), 3.24 (3H, s), 3.40-3.50 (4H, m), 3.84 (2H, m), 4.55-4.70 (1H, m), 4.63 (1H, d, J=8.3 Hz), 5.52 (1H, s), 6.87 (2H, d, J= 7.7 Hz), 7.0-7.4 (8H, m), 7.78 (1H, d, J=8.5 Hz), 8.94 (1H, d, J=8.3 Hz), 9.33 (1H, s).

Example 9

(3S, 4S)-3-{2S-2-[2-[2-(2-methoxyethoxy)ethoxy]acetamido]-2-benzyl-acetamido}-4-phenoxy-azetidin-2-one hydrochloride salt

By a similar method as described in example 8, the title compound is obtained by reacting 2-[2-(2-methoxyethoxy)ethoxy]acetic acid and (3S, 4S)-3-(2S-2-amino-2-benzyl-acetamido)-4-phenoxy-azetidin-2-one.

Yield: 96 %

^{1}H-NMR (DMSO-d_6), δ (ppm): 2.70-3.20 (2H, m), 3.23 (3H, s), 3.40-3.60 (8H, m), 3.84 (2H, m), 4.50-4.65 (1H, m), 4.65 (1H, d, J=8.3 Hz), 5.52 (1H, s), 6.87 (2H, d, J= 7.8 Hz), 7.0-7.4 (8H, m), 7.77 (1H, d, J=8.5 Hz), 8.92 (1H, d, J=8.3 Hz), 9.33 (1H, s).

Example 10

(3S, 4SR)-3-{2S-2-[2-(2-methoxyethoxy)acetamido]-2-cyclohexylmethyl-acetamido}-4-[4-(2S-2-amino-2-carboxy-ethyl)-phenoxy]-azetidin-2-one

To a solution of 2-(2-methoxyethoxy)acetic acid (670 mg, 5 mmole) and HOBt (675 mg, 5 mmole) in THF (10 ml), DCC (1.03 g, 5 mmole) in THF (10 ml) is added. The reaction mixture is stirred at room temperature for 1 hr and then cooled with an ice bath. The resulting DCU is removed by filtration. (3S, 4S)-3-(2S-2-amino-2-cyclohexyl methyl -acetamido)-4-acetoxy-azetidin-2-one trifluoroacetic acid salt (2.05 g, 5 mmole) in THF (10 ml) and triethylamine (555 mg, 5.5 mmole) are added at 0 °C and the resulting mixture is stirred at temperture from 0 °C to room temperature overnight. After removal of solvent, the residue is dissolved in ethyl acetate, washed with saturated $NaHCO_3$ solution, water, brine and dried over sodium sulfate. After removal of solvent, the solid is washed with ether and 1.5 g of (3S, 4S)-3-{2S-2-[2-(2-methoxyethoxy)acetamido]-2-cyclohexyl methyl -acetamido}-4-acetoxy-azetidin-2-one is obtained.

To a solution of 4-(2S-2-benzyloxycarbonylamino-2-diphenylmethoxycarbonyl-ethyl)-phenol (1.8 g, 3.75 mmole) in THF (20 ml), H_2O (5 ml) and 1N NaOH (3.25 ml, 3.25 mmole), (3S, 4S)-3-{2S-2-[2-(2-methoxyethoxy)acetamido]-2-cyclohexylmethyl -

acetamido}-4-acetoxy-azetidin-2-one (1.03 g, 2.5 mmole) in THF (20 ml) is added at 0 °C. The mixture is stirred at 0 °C for 1 hr and room temperature for 30 min. After removal of solvent, the residue is dissolved in ethyl acetate, washed with water, brine and dried over sodium sulfate. After removal of solvent, the residue is purified by silica gel column chromatography using hexane-ethyl acetate as eluent and 1.25 g of (3S, 4RS)-3-{2S-2-[2-(2-methoxyethoxy)acetamido]-2-cyclohexylmethyl -acetamido}-4-[4-(2S-2-benyloxycarbonylamino-2-diphenylmethoxycarbonyl-ethyl)-phenoxy]-azetidin-2-one is obtained.

(3S, 4RS)-3-{2S-2-[2-(2-methoxyethoxy)acetamido]-2-cyclohexylmethyl -acetamido}-4-[4-(2S-2-benzyloxycarbonylamino-2-diphenylmethoxycarbonyl-ethyl)-phenoxy]-azetidin-2-one (600 mg, 0.72 mmole) is hydrogenated with 800 mg of 10 % palladium on activated carbon in ethyl acetate (20 ml) at 50 psi hydrogen pressure at room temperature for 3 hrs. The solid is filterred, washed with ethyl acetate and extracted with a mixture of water/acetonitrile (1:1). After removal of solvent under vacuum and lyophilization, 320 mg of the title compound is obtained as white solid. Yield: 83 %; m.p.: 158-160 °C (dec.)

^{1}H-NMR (DMSO-d_6), δ (ppm): 0.70-1.80 (13H, m), 2.70-2.90 (1H, m), 3.00-3.20 (1H, m), 3.30 (3H, s), 3.35-3.40 (3H, br), 3.45-3.65 (4H, m), 3.91 (0.8H, s), 3.97 (1.2H, s), 4.30-4.50 (1H, m), 4.68 (0.6H, d, J=8.4 Hz), 5.35 (0.4H, dd, J=9.1 & 3.6 Hz), 5.52 (0.6H, s), 5.69 (0.4H, d, J=3.6 Hz), 6.70-6.90 (2H, m), 7.10-7.25 (2H, m), 7.70 (0.4H, d, J=8.7 Hz), 7.88 (0.6H, d, J=8.1 Hz), 8.85 (0.4H, d, J=9.3 Hz), 8.96 (0.6H, d, J=8.4 Hz), 9.1-9.5 (1H, br).

Example 11

(3S, 4S)-3-[2S-2-(4-acetimidoylamino butanoyl)-amino-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one hydrochloride salt

A reaction mixture of (3S, 4S)-3-[2S-2-(4-aminobutanoyl)-amino-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one triflouroacetic acid salt (100 mg, 0.19 mmole), ethylacetimidate hydrochloride (26 mg, 0.21 mmole) and triethylamine (58 mg, 0.58

mmole) in DMF (3 ml) is stirred at room temperature overnight and evaporated to dryness in vacuum. The residue is dissolved in water/acetonitrile and pH is adjusted to 1 by adding 1N HCl and purified by reversed-phase TLC using CH3CN/H2O (3:7) containing 0.5 M NH_4OAc as an eluent and 50 mg of the title compound is obtained.
Yield: 60 %; m.p.: 68-70 °C
^{1}H-NMR (DMSO-d_6), δ (ppm): 1.5-1.9 (5H, m), 2.05-2.3 (2H, m), 2.70-3.15 (4H, m), 3.2-3.9 (3H, br), 4.4-4.6 (1H, m), 4.64 (1H, d, J=8 Hz), 5.60 (1H, s), 6.87 (2H, d, J=8 Hz), 7.0-7.4 (8H, m), 9.0-9.7 (3H, m).

Example 12

(3S, 4S)-3-[2S-2-(5-acetimidoylamino pentanoyl)-amino-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one hydrochloride salt

By a similar method as described in example 11, the title compound is obtained from (3S, 4S)-3-[2S-2-(5-aminopentanoyl)-amino-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one triflouroacetic acid salt and ethylacetimidate hydrochloride
Yield: 54 %; m.p.: 68-70 °C
^{1}H-NMR (DMSO-d_6), δ (ppm): 1.2-1.6 (4H, m), 1.75 (3H, s), 2.0-2.2 (2H, m), 2.7-3.2 (4H, m), 4.4-4.6 (1H, m), 4.64 (1H, d, J=8.0 Hz), 5.58 (1H, s), 6.87 (2H, d, J=8 Hz), 7.0-7.4 (8H, m), 8.52 (1H, m), 8.96 (1H, d, J=8.5 Hz), 9.13 (1H, m), 9.47 (1H, d, J=8.5 Hz), 8.7-9.7 (2H, br).

Example 13

(3S, 4SR)-3-[2S-2-(6-guanidino hexanoyl)amino-2-cyclohexylmethyl-acetamido]-4-(3-carboxy-phenoxy)-azetidin-2-one

To a solution of 6-(N,N'-dibenzyloxycarbonylguanidino) hexanoic acid (1.16 g, 2.64 mmole) and 1-hydroxybenzotriazole (356 mg, 2.64 mmole) in THF (10 ml), DCC (544 mg, 2.64 mmole) is added. The reaction mixture is stirred at room temperature for 1 hr and cooled with an ice bath. the resulting DCU is removed by filtration. (3S, 4S)-3-(2S-2-amino-2-cyclohexylmethyl-acetamido)-4-acetoxy-azetidin-2-one trifluoroacetic acid salt (743 mg, 1.76 mmole) and triethylamine (267 mg, 2.64 mmole) are added at 0

°C and stirred at room temperature for 6 hrs. After removal of solvent, the residue is dissolved in ethyl acetate, washed with saturated $NaHCO_3$ solution, water, brine and dried over sodium sulfate. After removal of solvent, the residue is purified by silica gel column chromatography using hexane-ethyl acetate as eluent and 1.28 g of (3S, 4S)-3-[2S-2-(6-N,N'-dibenzyloxycarbonylguanidino hexanoyl) amino-2-cyclohexylmethyl-acetamido]-4-acetoxy-azetidin-2-one is obtained.

To a solution of 3-(diphenylmethoxycarbonyl) phenol (228 mg, 0.75 mmole) in THF (5 ml) and 1N NaOH (0.6 ml, 0.6 mmole), (3S, 4S)-3-[2S-2-(6-N,N'-dibenzyloxycarbonylguanidino hexanoyl) amino-2-cyclohexylmethyl-acetamido]-4-acetoxy-azetidin-2-one (365 mg, 0.5 mmole) in THF (5 ml) and H_2O (1 ml) is added at 0 °C. The mixture is stirred at 0 °C for 1 hr and then at room temperature for 1 hr. After removal of solvent, the residue is dissolved in ethyl acetate, washed with water, brine and dried over sodium sulfate. After removal of solvent, the residue is purified by silica gel column chromatography using hexane-ethyl acetate as eluent. 240 mg of (3S, 4SR)-3-[2S-2-(6-N,N'-dibenzyloxycarbonylguanidino hexanoyl) amino-2-cyclohexylmethyl-acetamido]-4-(3-diphenylmethoxycarbonyl-phenoxy)-azetidin-2-one is obtained.

(3S, 4SR)-3-[2S-2-(6-N,N'-dibenzyloxycarbonylguanidino hexanoyl) amino-2-cyclohexylmethyl-acetamido]-4-(3-diphenylmethoxycarbonyl-phenoxy)-azetidin-2-one (240 mg, 0.11 mmole) is hydrogenated with 10 % palladium on activated carbon in ethyl acetate (10 ml)/THF (10 ml) at 50 psi hydrogen pressure at room temperature for 4 hrs. The catalyst is filtered off. After removal of solvent, the solid is washed with ethyl acetate and ether and 50 mg of the title compound is obtained.

Yield: 38 %; m.p.: 98-100 °C

^{1}H-NMR (DMSO-d_6), δ (ppm): 0.7-1.8 (19H, m), 2.15-2.30 (2H, m), 3.0-3.20 (2H, m), 3.25-3.45 (4H, m), 4.4-4.6 (1H, m), 4.80 (0.6H, d, J=9.1 Hz), 5.30 (0.4H, m), 5.52 (0.6H, s), 5.70 (0.4H, d, J=3.9 Hz), 6.9-7.0 (1H, m), 7.2-7.35 (2H, m), 7.45-7.60 (1H, m), 8.05 (0.4H, d, J=9 Hz), 8.30 (0.6H, d, J=8.9 Hz), 8.45 (0.4H, d, J=9.1 Hz), 8.80 (0.6H, d, J=9.3 Hz), 9.19 (0.6H, s), 9.33 (0.4H, s), 9.57 (0.6H, m), 10.80 (0.4H, m).

Example 14

(3S, 4S)-3-(2S-2-(2-naphthyloxy)acetamido-2-benzyl-acetamido)-4-phenoxy-azetidin-2-one

To a suspension of (2-naphthyloxy)acetic acid (6.06 g, 0.03 mol) in dry DCM (150 ml), at 0 °C, drops of DMF were added (8 drops). Then, oxalyl chloride (3.14 ml, 0.036, 1.2 eq.) was added dropwise and the reaction mixture was stirred for 30 min. at 0 °C and 1.5 h at r.t. DCM and the excess of oxalyl chloride were evaporated to dryness, then chloroform was added and the solvent concentrated to dryness to ensure complete removal of the oxalyl chloride. The solution resulting from dissolving the greenish oily residue in THF (120 ml) was mixed with another one formed by dissolving the L-phenylalanine (4.95 g, 0.03 mol) in a solution of NaOH (3.6 g, 0.09 mol, water 350 ml). The mixture was stirred at 0 C for 4 hs. THF was removed under vacuo and subsequently, the remaining aqueous solution was acidified by adding 1 N sulfuric acid solution until the pH was about 2. The aqueous solution was extracted with chloroform (2 x 300 ml), and washing the extracts with 0.5 N sulfuric acid and brine. After concentration, 2S-2-(2-naphthyloxy) acetamido-2-benzyl-acetic acid separated out as a white solid, which was filtered and washed with cold ethyl acetate.

Yield: 30 %.

^{1}H-NMR (DMSO-d6), δ (ppm): 8.38 (1H, d, J: 8.2 Hz), 7.84 (2H, d, J: 9.4 Hz), 7.73 (1H, d, J: 7.9 Hz), 7.40 (2H, m), 7.20 (7H, m), 4.60 (2H, s), 4.54 (1H, m), 3.13 (1H, dd, J: 4.8, 13.8 Hz), 3.0 (1H, dd, J: 9.3, 13.8 Hz).

The title compound was obtained from (3S, 4S)-3-amino-4-phenoxy-azetidin-2-one (82 mg, 0.5 mmol) following a well established coupling procedure with DCC (103 mg, 0.5 mmol), HOBt (68 mg, 0.5 mmol) and 2S-2-(2-naphthyloxy)acetamido-2-benzyl-acetic acid (175 mg, 0.5 mmol) in a mixture of ethyl acetate/chloroform (10 ml/10 ml). The solid that was formed during the reaction was separated by filtration. The solid was

re-dissolved and purified by silica gel chromatography, using a mixture of ethyl acetate/hexane (7/3), ethyl acetate, and ethyl acetate/MeOH (9/1) as eluant. The title compound (138 mg) was obtained as a white solid.

Yield: 54 %.

^{1}H-NMR (DMSO-d6), δ (ppm): 9.35 (1H, s), 8.97 (1H, d, J: 8.3 Hz), 8.35 (1H, d, J: 8.2 Hz), 7.84 (2H, d, J: 9.4 Hz), 7.74 (1H, d, J: 8.0 Hz), 7.50-7.10 (11H, m), 7.00 (1H, t, J: 7.2 Hz), 6.86 (2H, d, J: 7.9 Hz), 5.51 (1H, s), 4.68 (1H, d, J: 8.0 Hz), 4.61 (2H, s), 4.60 (1H, m), 3.05 (2H, m).

Example 15

(3S, 4S)-3-[2S-2-(2-naphthyloxy)acetamido-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one

To a suspension of (2-naphthyloxy)acetic acid (4.04g, 0.02 mol) in dry DCM (150 ml), at 0 C, drops of DMF (6 drops) were added. Then oxalyl chloride (2.09 ml, 0.024 mol, 1.2 eq.) was dropwise added and the reaction mixture was stirred for 30 min. at 0 C and 1.5 hs at r.t. DCM and the excess of oxalyl chloride were evaporated to dryness, then chloroform was added and the solvent concentrated to dryness against to ensure complete removal of the oxalyl chloride. The solution resulting from dissolving the greenish oily residue in THF (80 ml) was mixed with another one formed by dissolving the (S)(-)-α- aminocyclohexane propionic acid hydrate (3.43 g, 0.02 mol) in a solution of NaOH (2.4 g, 0.06 mol, in 230 ml of water). The mixture was stirred for 4 hs at 0 C. THF was removed under vacuo and the remaining aqueous solution was acidified by adding 1N sulfuric acid solution until pH 2 and extracted with chloroform (2 x 300 ml) and the combined extracts washed with brine. After concentration, 2S-2-(2-naphthyloxy)acetamido-2-cyclohexylmethyl-acetic acid separated out as a solid which was separated by filtration and washed with ethyl ether.

Yield: 56 %.

^{1}H-NMR (DMSO-d6), δ (ppm): 8.38 (1H, d, J: 8.0 Hz), 7.80 (3H, m), 7.35 (4H, m), 4.69 (2H, m), 4.35 (1H, m), 1.80-1.40 (7H, m), 1.30-0.70 (6H, m).

The title compound was obtained from (3S, 4S)-3-amino-4-phenoxy-azetidin-2-one (82 mg, 0.5 mmol) following a well established coupling procedure with DCC (103 mg, 0.5 mmol), HOBt (68 mg, 0.5 mmol) and 2S-2-(2-naphthyloxy)acetamido-2-cyclohexylmethyl-acetic acid (178 mg, 0.5 mmol) in a mixture of ethyl acetate/chloroform (10 ml/10 ml). The solid was separated by filtration (DCU) and the filtrate was purified by silica gel column. The tittle compound (210 mg) was obtained as a foam.

Yield: 81 %

^{1}H-NMR (DMSO-d6), δ (ppm): 9.32 (1H, s), 8.85 (1H, d, J: 8.5 Hz), 8.30 (1H, d, J: 8.3 Hz), 7.85 (3H, m), 7.50-7.25 (6H, m), 6.99 (1H, t, J: 7.3 Hz), 6.85 (2H, d, J: 8.0 Hz), 5.53 (1H, s), 4.70 (3H, m), 4.40 (1H, m), 1.70-1.40 (7H, m), 1.25-1.30 (6H, m).

Example 16

(3S, 4S)-3-[2S-2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one

(3S, 4S)-3-(2S-2-amino-2-cyclohexylmethyl-acetamido)-4-phenoxy-azetidin-2-one (163 mg, 0.494 mmole) and 2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetic acid (151 mg, 0.494 mmole) were coupled following a well established procedure with DCC (102 mg, 0.494 mmole) and HOBt (68 mg, 0.494 mmole) in pure chloroform. The solid was separated by filtration and the filtrate was purified by silica gel chromatography, using ethyl acetate /hexane as eluent. The title compound (190 mg) was obtained as a white solid.

Yield: 63 %; m.p.: 162-164 °C.

^{1}H-NMR (DMSO-d6), δ (ppm): 9.31 (1H, s), 8.74 (1H, m), 7.97 (1H, m), 7.45 (1H, d, J: 8.0 Hz), 7.34 (7H, m), 7.01 (1H, t, J: 7.3 Hz), 6.86 (2H, d, J: 8.2 Hz), 5.50 (1H, s), 5.06 (1H, AB system, d, J: 12.7 Hz), 4.98 (1H, AB system, d, J: 12.7 Hz), 1.80-0.80 (26H, m).

Example 17

(3S, 4S)-3-[2S-2-(2S-2-amino-2-cyclohexylmethyl-acetamido)-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one hydrochloride salt

The title compound was obtained from hydrogenolysis of (3S, 4S)-3-[2S-2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one (80 mg, 0.132 mmol.) with 10% Pd/C (150 mg) in ethyl acetate (15 ml) at 50 psi for 3 hrs. The catalyst was separted by filtration through Celite and the filtrate concentrated to dryness. The residue was redissolved in acetonitrile (5 ml) and 1ml of water containing 4 drops of cc HCl was added. The solution was freeze dried to obtain the title compound as a white powder.
Yield: 100 %; m.p.: 165-170 °C.
^{1}H-NMR (DMSO-d6), δ (ppm): 9.34 (1H, s), 9.00 (1H, d, J: 8.0 Hz), 8.7 (1H, d, J: 8.0 Hz), 8.15 (3H, bs), 7.32 (2H, t, J: 7.5 Hz), 7.02 (1H, t, J: 7.2 Hz), 6.86 (2H, d, J: 8.0 Hz), 5.50 (1H, s), 4.70 (1H, m), 4.35 (1H, m), 3.85 (1H, m), 1.8-0.7 (26H, m).

Example 18

(3S, 4S)-3-[2S-2-(2S-2-t-butyloxycarbonylamino-2-benzyloxymethyl-acetamido)-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one

2S-2-(2S-2-t-butyloxycarbonylamino-2-benzyloxymethyl-acetic acid (172 mg, 0.58 mmol) was reacted overnight with (3S, 4S)-3-(2S-2-amino-2-cyclohexylmethyl-acetamido)-4-phenoxy-azetidin-2-one (191 mg, 0.58 mmol) under a standard coupling condition with DCC (120 mg, 0.58 mmol), HOBt (79 mg, 0.58 mmol) in chloroform (10 ml). The solids were separated by filtration and the filtrate was concentrated. The residue was purified through a silica gel column, using a mixture of ethyl acetate/hexane as eluant. The title compound (252 mg) was obtained as a foam.
Yield: 71 %.
^{1}H-NMR (DMSO-d6), δ (ppm): 9.29 (1H, s), 8.68 (1H, d, J: 8.3 Hz), 8.07 (1H, d, J: 7.9 Hz), 7.30 (7H, m), 7.05 (2H, m), 6.84 (2H, d, J: 7.8 Hz), 5.48 (1H, s), 4.56 (1H, d, J: 8.3 Hz), 4.46 (2H, s), 4.30 (2H, m), 3.59 (2H, m), 1.80-0.8 (13H, m).

Example 19

(3S, 4S)-3-[2S-2-(2S-2-amino-2-benzyloxymethyl-acetamido)-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one hydrochloride salt

(3S, 4S)-3-[2S-2-(2S-2-t-butyloxycarbonylamino-2-benzyloxymethyl-acetamido)-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one (200 mg, 0.33 mmol) was dissolved in a mixture of TFA/DCM (1 ml/2 ml) at 0 C and the mixture stirred for 1 h and 45 min. at r.t. Then, the solvents were removed under vacuo and the residue dissolved in ethyl acetate, washed with sat. sol. of $NaHCO_3$ and brine. After drying over sodium sulfate, the solvent was evaporated to get the free amine as a glass (147 mg). To a solution formed by dissolving 45 mg of the crude (0.0885 mmol) in acetonitrile (5 ml), water (1 ml) containing four drops of cc HCl was added. The mixture was freeze dried to obtain the title compound (149 mg) as a white fluffy solid.

Yield: 100 %; m.p.: 140-142 °C.

^{1}H-NMR (DMSO-d6), δ (ppm): 9.35 (1H, s), 8.97 (1H, d, J: 8.4 Hz), 8.75 (1H, d, J: 8.4 Hz), 8.27 (3H, bs), 7.33 (7H, m), 7.01 (1H, t, J: 7.2 Hz), 6.86 (2H, d, J: 7.8 Hz), 5.51 (1H, s), 4.65 (1H, d, J: 7.8 Hz), 4.52 (2H, s), 4.40 (1H, m), 4.08 (1H, m), 3.75 (2H, m), 1.80-0.80 (13 H, m).

Example 20

(3S, 4S)-3-[2S-2-(2S-2-acetamido-2-benzyloxymethyl-acetamido)-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one

(3S, 4S)-3-[2S-2-(2S-2-amino-2-benzyloxymethyl-acetamido)-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one (100 mg, 0.196 mmol), obtained according to the procedure above described, was dissolved in chloroform (5 ml) and cooled at 0 C. To this solution, acetyl chloride (17 µl, 0.236 mmol, 1.2 eq.) and ethyl acetate (33 µl, 0.236 mmol, 1.2 eq.) were added. The reaction was carried out at that temperature for 1 h, and 1 h at r.t. The solution was diluted with chloroform (50 ml) and washed with water. After drying over sodium sulfate, the solvent was evaporated under vacuo. The crude

residue was purified by preparative TLC, using ethyl acetate/hexane (6/4) as mobile phase. The title compound (67 mg) was obtained as a foam.
Yield: 62 %.
^{1}H-NMR (DMSO-d6), δ (ppm): 9.29 (1H, s), 8.57 (1H, d, J: 8.1 Hz), 8.22 (1H, d, J: 7.4 Hz), 8.14 (1H, d, J: 8.0 Hz), 7.30 (7H, m), 7.01 (1H, t, J: 7.2 Hz), 6.84 (2H, d, J: 8.1 Hz), 5.48 (1H, s), 4.56 (1H, d, J: 8.1 Hz), 4.47 (2H, s), 4.32 (1H, m), 3.60 (2H, d, J: 5.5 Hz), 1.88 (3H, s), 1.80-0.80 (13H, m).

Example 21

(3S, 4S)-3-[2S-2-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one

2S-2-benzyloxycarbonylamino-2-benzyl-acetic acid (194 mg, 0.65 mmol) and (3S, 4S)-3-(2S-2-amino-2-cyclohexylmethyl-acetamido)-4-phenoxy-azetidin-2-one (214 mg, 0.65 mmol) were reacted under coupling condition with DCC (165 mg, 0.65 mmol) and HOBt (108 mg, 0.65 mmol) in chloroform (10 ml), overnight. The solids were separated by filtration and purified by silica gel column, using ethyl acetate/hexane as eluant. 310 mg of the title compound was obtained.
Yield: 50 %.
^{1}H-NMR (DMSO-d6), δ (ppm): 9.32 (1H, s), 8.77 (1H, d, J: 8.4 Hz), 8.21 (1H, d, J: 8.4 Hz), 7.54 (1H, d, J: 8.6 Hz), 7.40-7.10 (7H, m), 7.03 (1H, t, J: 7.3 Hz), 6.87 (2H, d, J: 7.90 Hz), 5.53 (1H, s), 4.67 (1H, d, J: 8.3 Hz), 4.35 (2H, m), 3.00 (1H, m), 2.75 (1H, m), 1.80-0.80 (13H, m).

Example 22

(3S, 4S)-3-[2S-2-(2S-2-amino-2-benzyl-acetamido)-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one hydrochloride salt

(3S, 4S)-3-[2S-2-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-2-cyclohexyl methyl-acetamido]-4-phenoxy-azetidin-2-one (impurified with DCU) (280 mg, 0.40 mmol) was hydrogenated at 50 psi in ethanol (20 ml), using Pd/C (10%) (270 mg)

as a catalyst, during 3 hs. The catalyst was separted by filtration through Celite and the filtrate was concentrated, and purified by preparative TLC, using ethyl acetate/MeOH (9/1) as a mobile phase. The solid so obtained was proved to be a mixture of the free amine and DCU in a 2:1 ratio (55 mg, Yield ~ 23 %). To the solution formed by dissolving the mentioned solid in acetonitrile (5 ml), 1 ml of water containing 4 drops of cc HCl was added. The mixture was freeze dried to get a white solid (59 mg).
Yield: 100 %.
^{1}H-NMR (DMSO-d6), δ (ppm): 9.30 (1H, s), 8.79 (1H, d, J: 8.3 Hz), 8.10 (1H, d, J: 8.3 Hz), 7.40-7.15 (7H, m), 7.03 (1H, t, J: 7.3 Hz), 6.89 (2H, d, J: 7.7 Hz), 5.55 (1H, s), 4.65 (1H, d, J: 8.3 Hz), 4.35 (1H, m), 3.45 (1H, m), 2.95 (1H, dd, J: 4.4, 13.4 Hz), 2.63 (1H, dd, J: 8.3, 13.4 Hz), 1.80-0.80 (13H, m).

Example 23

(3S, 4S)-3-[2(S)-2-(biotinoyl)amino-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one

A solution of succinimido biotinate (103mg, 0.25 mmol) in dry DMF(7ml) under nitrogen was treated with (3S, 4S)-3-(2S-2-amino-2-benzyl-acetamido)-4-phenoxy-azetidin-2-one (82 mg, 0.25 mmol) and stirred at r.t. for 4 hrs. The reaction mixture was concentrated *in vacuo* and the slurry obtained was treated with a mixture of ether:iso-PrOH(2:1). The solid separated was filtered, washed with ether and dried to give an off white solid. Purification of the above crude product over reverse phase preparative TLC plates by using a mixture of CH_3CN:H_2O (9:1) as developing solvent followed by extraction and lyophilization gave the title compound (119 mg).
Yield: 86%.
^{1}H NMR(DMSO-d_6), δ (ppm): 1.08-1.70(m, 7H, btn-H), 2.00-2.10(m, 2H, btn-H), 2.60-3.30(m, 5H, btn and CH_2Ph), 4.04-4.20(m, 2H, C<u>H</u>CH_2Ph and btn-H), 4.47-4.58(m, 1H, btn ring-H), 4.65(d, 1h, J=8.3Hz, C_3H), 5.53(s, 1H, C_4H), 6.37, 6.41 and 6.45(2s, 2H, N<u>H</u>CON<u>H</u>), 6.85-7.36(m, 10H, ar), 8.23(d, 1H, J=8.2Hz, NH), 8.95(d, 1H, J=10.0, NH), 9.33(s,1H, ring NH).

Example 24

(3S, 4S)-3-[2S-2-(2-carboxymethoxy acetamido)-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one

A solution of (3S, 4S)-3-[2S-2-amino-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one trifluoroacetate salt (106 mg, 0.24 mmol) in dry DMF (5ml) under nitrogen was treated with TEA (26 mg, 0.253 mmol) at r.t. Within 5 min. was added diglycolic anhydride(31 mg, 0.265 mmol) and the reaction mixture was allowed to stir for 1h.. DMF was removed *in vacuo* and the gummy mass was taken in EtOAc (25ml), washed with 1N HCl, water, brine and dried over Na_2SO_4. Evaporation of the volatile solvents followed by purification by reverse phase silica gel preparative TLC (Analtech@ RPSF plates), using CH_3CN:H_2O(85:15) as developing solvent and lyophilization gave the title compound (26 mg).

Yield: 24%; m.p.: 143-147 °C

^{1}H NMR(DMSO-d_6), δ (ppm): 2.51-2.87(m, 2H, CH_2Ph), 3.63(s, 2H, $COCH_2O$), 3.80(s, 2H, $COCH_2O$), 4.46-4.59(m, 1H, C<u>H</u>CH_2Ph), 5.35(dd, 1H, J= 3.0Hz and 7.5Hz, C_3H), 5.75(d, 1H, J= 3.7Hz, C_4H), 6.92-7.34(m, 11H, aromatic and NH), 8.85(d, 1H, J= 8.0Hz, NH), 9.31(s, 1H, NH), 10.0(br.s, 1H, COOH).

Example 25

(3S,4S)-3-[2S-2-(1-benzyloxycarbonylaminocyclopropyl-1-carbonyl)amino-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one

A mixture of 1-(benzyloxycarbonyl)amino-cyclopropane-1-carboxylic acid(143mg, 0.61mmol), (3S,4S)-3-[2S-2-amino-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one (125mg, 0.61mmol), DCC(125mg, 0.61mmol) and 1-HOBt(82mg, 0.61mmol) in dry DMF was stirred under nitrogen at r.t. for 6 hrs. The suspension obtained was filtered and the filtrate was evaporated *in vacuo* to give a gummy mass. The gummy mass was purified over silica gel column chromatography using a mixture of hexanes:EtOAc(3:7) to give the title compound (318 mg).

Yield: 78%; m.p.: 109-112 °C

^{1}H NMR(DMSO-d_6), δ (ppm): 0.80-1.80(m, 17H, Chx, CH_2Chx and cp-H), 4.32-4.45(m, 1H, C<u>H</u>CH_2Chx), 4.70(d,1H, J= 8.0Hz, C_3H), 5.04(ABq, 2H, J= 7.9Hz and 12.6Hz, CH_2O), 5.54(s, 1H, C_4H), 6.86-7.34(m, 10H, ar), 7.73(d, 1H, J= 8.5Hz, NH), 7.95(s, 1H, cp-NH), 8.70(d, 1H, J= 8.0Hz, NH), 9.32(s, 1H, ring-NH).

Example 26

(3S,4S)-3-[2S-2-(4-pyridylthio)acetamido-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one

The title compound was synthesized according to the procedure explained for example 25, and by the reaction of (4-pyridylthio) acetic acid with (3S,4S)-3-[2S-2-amino-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one.

Yield: 33%; m.p.: 124-128 °C

^{1}H NMR(DMSO-d_6), δ (ppm): 0.70-1.60(m, 13H, Chx and CH_2Chx), 3.86(ABq, 2H, J= 10.9 and 15.1Hz, SCH_2), 4.30-4.40(m, 1H, C<u>H</u>CH_2Chx), 4.66(d, 1H, J= 8.3Hz, C_3H), 5.52(s, 1H, C_4H), 6.84-7.34(m, 7H, ar), 8.35(dd, 2H, J= 1.5Hz and 3.3Hz, py-H), 8.53(d, 1H, J= 8.0Hz, NH), 8.90(d, 1H, J= 8.4Hz, NH) 9.33(s, 1H, ring-NH)

Example 27

(3S,4S)-3-[2S-2-(1-benzyloxycarbonylaminocyclohexyl-1-carbonyl)amino-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one

The title compound was synthesized according to the procedure explained for example 25, and by the reaction of 1-(benzyloxycarbonyl)amino-cyclohexane-1-carboxylic acid with (3S,4S)-3-[2S-2-amino-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one in presence of DCC and HOBt in DMF.

Yield: 59%; m.p.: 104-106 °C

^{1}H NMR(DMSO-d_6), δ (ppm): 0.75-0.90(m, 23H, CH_2Chx and Chx-H), 4.39-4.40(m, 1H, C<u>H</u>CH_2Chx), 4.67(d, 1H, J=8.4Hz, C_3H), 4.98(ABq, 2H, J= 2.0Hz, O<u>C</u>H_2), 5.53(s, 1H, C_4H), 6.85-7.48(m, 10H, ar), 7.75(d, 1H, J=6.0Hz, NH), 8.48(d, 1H, J= 8.5Hz, NH), 9.33(s, 1H, ring-NH).

Example 28

(3S,4S)-3-[2S-2-carboxymethylthio acetamido-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one sodium salt

A solution of TFA salt of (3S,4S)-3-[2S-2-amino-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one (123mg, 0.28mmol) in dry DMF(4ml) was treated with TEA(30mg, 0.3mmol) and stirred for 10 min. Thiodiglycolic anhydride(37mg, 0.28mmol) was added to the reaction mixture in one portion, stirred for 2 hrs and evaporated *in vacuo* to remove DMF. The crude product was taken in EtOAc, washed with water, brine solution, dried over Na_2SO_4 and evaporated. The solid obtained was suspended in distilled water(20ml), treated with $NaHCO_3$(47mg, 0.56mmol) and the clear solution was lyophilized. Futher purification of the product by reverse phase preparative TLC(Analtech@ RPSF plate), followed by lyophilization gave the title compound (85 mg).

Yield: 63%; m.p.: 152-154^{0}C

^{1}H NMR(DMSO-d_6), δ (ppm): 2.60-3.10(m, 2H, CH_2Ph), 4.40-4.50(m,1H, C<u>H</u>CH_2Ph), 5.35(dd, 1H, J= 2.0Hz, and 4.0 Hz, C_3H), 5.73(d, 1H, J= 3.8 Hz, C_4H), 6.93-7.34(m, 10H, ar), 8.93(d, 1H, J= 9.2 Hz, NH), 9.28(s, 1H, ring-NH), 9.55(d, 1H, J= 8.1 Hz, NH)

Example 29

(3S,4S)-3-[2S-2-(3,4-methylenedioxy phenoxy) acetamido-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one

A mixture of 3,4-methylenedioxy phenoxy acetic acid (46mg, 0.234mmol), DCC(48mg, 0.234mmol), and HOBt (32mg, 0.234) in dry DMF(4ml) was stirred under nitrogen for 1 hr. A solution of TFA salt of (3S,4S)-3-[2S-2-amino-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one in DMF (1ml) was treated with TEA(25mg, 0.246mmol) and within 5 min. was added to the reaction mixture. After stirring for 4 hrs. the resulting suspension was filtered and the filtrate was evaporated *in vacuo* to give a solid. The crude solid was dissolved in EtOAc, washed with water, sat. $NaHCO_3$, brine, dried over

Na_2SO_4 and evaporated *in vacuo* to give a solid. The above solid was triturated with a mixture of ether:EtOAc:MeOH(0.5:8.5:1.0), filtered, washed with ether and air dried to give the title compound (80 mg).

Yield: 68%; m.p.: 198-199°C

^{1}H NMR(DMSO-D_6), δ (ppm): 2.70-3.00(m, 2H, CH_2Ph), 4.29(s, 2H, $COCH_2$), 4.55-4.70(m, 1H, C<u>H</u>CH_2Ph), 5.38(dd, 1H, J= 3.7 Hz and 5.6 Hz, C_3H), 5.76(d, 1H, J= 3.7 Hz, C_4H), 5.96(s, 2H, OCH_2O), 6.22(dd, 1H, J= 2.5 Hz and 6.0 Hz, ar), 6.56(d, 1H, J = 2.5 Hz, ar), 6.72-7.32(m, 11H, ar), 8.05(d, 1H, J= 8.7 Hz, NH), 9.02(d, 1H, J= 9.23 Hz, NH), 9.38(s, 1H, ring-NH)

<u>Example 30</u>

<u>(3S,4S)-3-{2(S)-2-[6-(biotinoylamino)hexanoyl]amino-2-benzyl-acetamido}-4-phenoxy-azetidin-2-one</u>

A solution of succinimido 6-biotinoylamino hexanoate (171mg, 0.48 mmol) and (3S,4S)-3-[2S-2-amino-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one (156mg, 0.48 mmol) in dry DMF(15ml) was stirred at r.t. for 4 hrs.. The reaction mixture was concentrated *in vacuo* and the slurry obtained was treated with a mixture of ether:iso-PrOH(2:1). The solid separated was filtered, washed with ether and dried to give an off white solid. The above solid was further purified by reverse phase silica gel preparative TLC (CH_3CN:H_2O/ 9:1) to give the pure compound after lyophilization (78 mg).

Yield: 25%.

^{1}H NMR(DMSO-d_6), δ (ppm): 1.00-1.62(m, 14H, aliph.H), 2.00-2.10(m, 4H, aliph-H), 2.60-3.15(m, 5H, aliph-H, and CH_2Ph), 4.10-4.16(m, 1H, C<u>H</u>CH_2Ph), 4.25-4.34(m, 1H, btn.ring-H), 4.44-4.56(m,1H, btn.ring-H), 4.64(d, 1H, J= 8.0Hz, C_3H), 5.52(s, 1H, C_4H), 6.37 and 6.44(2s, 2H, NHCONH), 6.84-7.32(m, 10H, ar), 7.36(t, 1H, J= 5.3Hz, CONHCH2), 8.22(d,1H, J= 8.0Hz, NH), 8.92(d, 1H, J= 8.0Hz, NH), 9.33(s, 1H, ring-NH).

<u>Example 31</u>

<u>(3S, 4SR)-3-[2S-2-(6-guanidino hexanoyl)-amino-2-cyclohexylmethyl-acetamido]-4-[4-(2S-2-amino-2-carboxy-ethyl)-phenoxy]-azetidin-2-one dihydrochloride</u>

To a solution of 4-(2S-2-benzyloxycarbonylamino-2-diphenylmethoxycarbonyl-ethyl)-phenol (291 mg, 0.616 mmole) in THF (5 ml) and 1N NaOH (0.5 ml, 0.5 mmole), (3S, 4S)-3-[2S-2-(6-N,N'-dibenzyloxycarbonylguanidino hexanoyl) amino-2-cyclohexylmethyl-acetamido]-4-acetoxy-azetidin-2-one (300 mg, 0.41 mmole) in THF (5 ml) and H_2O (1 ml) is added at 0 °C. The mixture is stirred at 0 °C for 1 hr and then at room temperature for 2 hr. After removal of solvent, the residue is dissolved in ethyl acetate, washed with water, brine and dried over sodium sulfate. After removal of solvent, the residue is purified by silica gel column chromatography using hexane-ethyl acetate as eluent. 200 mg of (3S, 4SR)-3-[2S-2-(6-N,N'-dibenzyloxycarbonylguanidino hexanoyl) amino-2-cyclohexylmethyl-acetamido]-4-[4-(2S-2-benzyloxycarbonylamino-2-diphenylmethoxycarbonyl-ethyl)-phenoxy]-azetidin-2-one is obtained.

(3S, 4SR)-3-[2S-2-(6-N,N'-dibenzyloxycarbonylguanidino hexanoyl) amino-2-cyclohexylmethyl-acetamido]-4-[4-(2S-2-benzyloxycarbonylamino-2-diphenylmethoxycarbonyl-ethyl)-phenoxy]-azetidin-2-one (200 mg, 0.17 mmole) is hydrogenated with 10 % palladium on activated carbon in ethyl acetate (10 ml)/THF (10 ml) and 1N HCl (0.5 ml, 0.5 mmole) at 50 psi hydrogen pressure at room temperature for 4 hrs. The solid is filtered, washed with ethyl acetate and then extracted with a mixture of water/acetonitrile (1:1). After removal of solvent under vacuum and lyophilization, 110 mg of the title compound is obtained.

Yield: 96 %

^{1}H-NMR (DMSO-d_6), δ (ppm): 0.7-1.8 (19H, m), 2.0-2.2 (2H, m), 2.9-3.3 (4H, m), 3.7-3.9 (1H, m), 4.2-4.5 (1H, m), 4.62 (0.6H, d, J=8.4 Hz), 5.30 (0.4H, m), 5.48 (0.6H, s), 5.63 (0.4H, d, J=3.5 Hz), 6.75-6.90 (2H, m), 7.10-7.30 (2H, m), 7.35-7.60 (4H, br), 7.8-8.5 (5H, m), 8.61 (0.4H, d, J=8.4 Hz), 8.89 (0.6H, d, J=8.4 Hz), 9.35 (1H, s)

Example 32

(3S, 4R)-3-[2S-2-(6-guanidino hexanoyl)amino-2-cyclohexylmethyl-acetamido]-4-(3-carboxy-phenoxy)-azetidin-2-one

To a solution of 3-(diphenylmethoxycarbonyl)-phenol (3.8 g, 12.3 mmole) in THF

(30 ml) and 1N NaOH (11.4 ml, 11.4 mmole), (3S, 4S)-3-benzyloxycarbonylamino-4-acetoxy-azetidin-2-one (2.3 g, 8.27 mmole) in THF (20 ml) is added at 0 °C. The mixture is stirred at 0 °C for 1 hr and then at room temperature for 1 hr. After removal of solvent, the residue is dissolved in ethyl acetate, washed with water, brine and dried over sodium sulfate. After removal of solvent, the residue is purified by silica gel column chromatography using hexane-ethyl acetate as eluent. 2.3 g of (3S, 4S)-3-benzyloxycarbonylamino-4-(3-diphenylmethoxycarbonyl-phenoxy)-azetidin-2-one (yield 55%) and 1.45 g of (3S, 4R)-3-benzyloxycarbonylamino-4-(3-diphenylmethoxycarbonyl-phenoxy)-azetidin-2-one (yield 33%) are obtained.

(3S, 4R)-3-benzyloxycarbonylamino-4-(3-diphenylmethoxycarbonyl-phenoxy)-azetidin-2-one (1.4 g, 2.68 mmole) is hydrogenated with 2.8 g of 10 % palladium on activated carbon in ethyl acetate (30 ml) at 50 psi hydrogen pressure at room temperature for 4 hrs. The solid is filtred, washed with ethyl acetate and then extracted with a mixture of water/acetonitrile (1:1). After removal of solvent under vacuum and lyophilization, 400 mg of (3S, 4R)-3-amino-4-(3-carboxy-phenoxy)-azetidin-2-one (yield 67%) is obtained as white solid.

To a solution of (3S, 4R)-3-amino-4-(3-carboxy-phenoxy)-azetidin-2-one (400 mg, 1.8 mmole) in acetone (30 ml), diazodiphenylmethane (340 mg, 1.8 mmole) in acetone (10 ml) is added at 0 °C. The reaction mixture is stirred at 0 °C for 2 hrs and room temperature overnight. After removal of solvent, the residue is purified by silica gel chromatography using hexane-ethyl acetate as eluent and 430 mg of (3S, 4R)-3-amino-4-(3-diphenylmethoxycarbonyl-phenoxy)-azetidin-2-one (yield 62%) is obtained as a white solid.

^{1}H-NMR (DMSO-d6), δ (ppm): 2.05-2.20 (2H, br), 4.36 (1H, m), 5.75 (1H, d, J=3.6 Hz), 7.04 (1H, s), 7.20-7.80 (14H, m), 9.00 (1H, s).

To a solution of 2S-2-(6-N,N'-dibenzyloxycarbonylguanidino hexanoyl) amino-2-cyclohexylmethyl-acetic acid (2.6 g, 4.37 mmole) and 1-hydroxybenzotriazole (590 mg, 4.37 mmole) in THF (25 ml), DCC (900 mg, 4.37 mmole) is added. The reaction mixture is stirred at room temperature for 2 hr and cooled with an ice bath. the resulting DCU is

removed by filtration. (3S, 4R)-3-amino-4-(3-diphenylmethoxycarbonyl-phenoxy)-azetidin-2-one (1.2 g, 3.08 mmole) is added at 0 °C and stirred at room temperature overnight. After removal of solvent, the residue is dissolved in ethyl acetate, washed with saturated $NaHCO_3$ solution, water, brine and dried over sodium sulfate. After removal of solvent, the residue is purified by silica gel column chromatography using hexane-ethyl acetate as eluent and 1.99 g of (3S, 4R)-3-[2S-2-(6-N,N'-dibenzyloxycarbonylguanidino hexanoyl)-amino-2-cyclohexylmethyl-acetamido]-4-(3-diphenylmethoxycarbonyl-phenoxy)-azetidin-2-one (yield 67%) is obtained.
^{1}H-NMR (DMSO-d_6), δ (ppm): 0.6-1.7 (19H, m), 1.9-2.1 (2H, m), 3.15-3.35 (2H, m), 4.20-4.40 (1H, m), 5.02 (2H, s), 5.20 (2H, s), 5.33 (1H, m), 5.84 (1H, d, J=3.5 Hz), 7.03 (1H, s), 7.15-7.80 (25H, m), 8.37 (1H, m), 8.59 (1H, d, J=9.1 Hz), 9.30 (1H, s), 11.60 (1H, s).

(3S, 4R)-3-[2S-2-(6-N,N'-dibenzyloxycarbonylguanidino hexanoyl) amino-2-cyclohexylmethyl-acetamido]-4-(3-diphenylmethoxycarbonyl-phenoxy)-azetidin-2-one (1.15 g, 1.19 mmole) is hydrogenated with 10 % palladium on activated carbon in ethyl acetate (50 ml) at 50 psi hydrogen pressure at room temperature for 2 hrs. The solid is filtered, washed with ethyl acetate, water and then extracted with a mixture of water/acetonitrile (1:1). After removal of solvent under vacuum and lyophilization, 490 mg of the title compound is obtained.
Yield: 78 %
^{1}H-NMR (DMSO-d_6), δ (ppm): 0.6-1.7 (19H, m), 2.1-2.3 (2H, m), 3.0-3.15 (2H, m), 4.4-4.6 (1H, m), 5.27 (1H, m), 5.70 (1H, d, J=3.9 Hz), 6.85-7.50 (8H, m), 8.04 (1H, d, J=8.3 Hz), 8.51 (1H, d, J=9.1 Hz), 9.36 (1H, s), 10.77 (1H, s).

Example 33

(3S, 4S)-3-[2S-2-(trans-2-phenylcyclopropane-1-carbonyl) amino-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one

By a similar method as described in example 16, the title compound is obtained by reacting trans-2-phenylcyclopropane-1-carboxylic acid with (3S, 4S)-3-(2S-2-amino-

2-cyclohexylmethyl-acetamido)-4-phenoxy-azetidin-2-one.

Yield: 30 %.

^{1}H-NMR (DMSO-d6), δ (ppm): 0.8-1.7 (15H, m), 1.95-2.1 (1H, m), 2.20-2.35 (1H, m), 4.25-4.45 (1H, m), 4.66 (1H, d, J=8.5 Hz), 5.52 (1H, s), 6.8-7.4 (10H, m), 8.37 (1H, d, J=8.3 Hz), 8.84 (1H, d, J=8.5 Hz), 9.30 (1H, s).

Example 34

(3S, 4S)-3-[2S-2-[6-(benzotriazol-1-yl-oxy) hexanoyl]amino-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one

To a solution of 6-bromohexanoic acid (1.19 g, 6.08 mmole), L-phenylalanine diphenylmethyl ester p-toluenesulfonate salt (2.55 g, 5.07 mmole) and PyBOP (2.64 g, 5.08 mmole) in DMF (15 ml), triethylamine (2.14 ml, 15.2 mmole) is added at 0 °C. The reaction mixture is stirred at room temperature overnight and then diluted with ethyl acetate (300 ml) and ether (100 ml), washed with saturated $NaHCO_3$ solution, water, brine and dried over sodium sulfate. After removal of solvent, the residue is purified by silica gel column chromatography using hexane-ethyl acetate as eluent and 600 mg of diphenylmethyl (2S)-2-(6-bromohexanoyl)amino-2-benzyl-acetate and 1.5 g of diphenylmethyl (2S)-2-[6-(benzotriazol-1-yl-oxy) hexanoyl]amino-2-benzyl-acetate are obtained.

Diphenylmethyl (2S)-2-[6-(benzotriazol-1-yl-oxy) hexanoyl]amino-2-benzyl-acetate (1.5 g, 2.67 mmole) is dissolved in a mixture of TFA/DCM (8 ml/8 ml) and anisole (0.5 ml) at 0 C and the mixture is stirred at room temperature for 1 hr . Then, After removal of the solvent under vacuo, the residue is triturated with ether and 1 g of (2S)-2-[6-(benzotriazol-1-yl-oxy) hexanoyl]amino-2-benzyl-acetic acid is obtained.

To a solution of (2S)-2-[6-(benzotriazol-1-yl-oxy) hexanoyl]amino-2-benzyl-acetic acid (250 mg, 0.63 mmole) and 1-hydroxybenzotriazole (85 mg, 0.63 mmole) in THF (5 ml), DCC (130 mg, 0.63 mmole) is added at 0 °C. The reaction mixture is stirred at room temperature for 1 hr and then cooled with an ice bath. The resulting DCU is removed by filtration. Then (3S, 4S)-3-amino-4-phenoxy-azetidin-2-one is added and

the resulting mixture is stirred at room temperature overnight. After removal of solvent, the residue is dissolved in ethyl acetate, washed with saturated $NaHCO_3$ solution, water, brine and dried over sodium sulfate. After removal of solvent, the solid is recrystallized from ethyl acetate /hexane and 250 mg of the title compound is obtained.
Yield: 71 %.
^{1}H-NMR (DMSO-d6), δ (ppm): 1.1-1.8 (6H, m), 2.11 (2H, t, J=6.8 Hz), 2.7-2.9 (1H, m), 3.0-3.15 (1H, m), 4.46 (2H, t, J=6.5 Hz), 4.4-4.6 (1H, m), 4.66 (1H, d, J=8.3 Hz), 5.52 (1H, s), 6.85 (2H, d, J=7.7 Hz), 7.0-7.4 (8H, m), 7.51 (1H, m), 7.64 (1H, m), 7.82 (1H, d, J=8.3 Hz), 8.08 (1H, d, J=8.3 Hz), 8.19 (1H, d, J=8.3 Hz), 8.88 (1H, d, J=8.3 Hz), 9.33 (1H, s).

Example 35

(3S, 4S)-3-[2-[6-(1,2,4-triazol-3-yl-amino) hexanoyl]amino-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one

A reaction mixture of diphenylmethyl (2S)-2-(6-bromohexanoyl)amino-2-benzyl-acetate (254 mg, 0.5 mmole), 3-amino-1,2,4-triazole (84 mg, 1 mmole) and KOH (56 mg, 1 mmole) in DMSO (5 ml) is stirred at room temperature for 3 hrs and then diluted with ethyl acetate, washed with water, brine and dried over sodium sulfate. After removal of solvent, the residue is purified by silica gel column chromatography using ethyl acetate-methanol as eluent and 180 mg of diphenylmethyl (2S)-2-[6-(1,2,4-triazol-3-yl-amino) hexanoyl] amino-2-benzyl-acetate (yield 70%) is obtained.

Diphenylmethyl (2S)-2-[6-(1,2,4-triazol-3-yl-amino) hexanoyl]amino-2-benzyl-acetate (300 mg, 0.58 mmole) is dissolved in a mixture of TFA/DCM (8 ml/8 ml) and anisole (0.5 ml) at 0 °C and the mixture is stirred at room temperature for 1 hr . Then, After removal of the solvent under vacuo, the residue is triturated with ether and 180 mg of (2S)-2-[6-(1,2,4-triazol-3-yl-amino) hexanoyl]amino-2-benzyl-acetic acid trifluoroacetic acid salt is obtained.

To a solution of (2S)-2-[6-(1,2,4-triazol-3-yl-amino) hexanoyl]amino-2-benzyl-acetic acid trifluoroacetic acid salt (180 mg, 0.39 mmole), (3S, 4S)-3-amino-4-phenoxy-

azetidin-2-one (71 mg, 0.4 mmole) and BOP (177 mg, 0.4 mmole) in DMF (4 ml), triethylamine (0.168 ml, 1.2 mmole) is added at 0 °C. The reaction mixture is stirred at room temperature overnight and then diluted with ethyl acetate (200 ml) and ether (100 ml), washed with saturated $NaHCO_3$ solution, water, brine and dried over sodium sulfate. After removal of solvent, the residue is purified by silica gel column chromatography using chloroform-methanol as eluent and 110 mg of the title compound is obtained.

Yield: 56 %.

^{1}H-NMR (DMSO-d6), δ (ppm): 1.0-1.7 (6H, m), 2.06 (2H, m), 2.7-2.85 (1H, m), 2.95-3.1 (1H, m), 3.70-3.85 (2H, m), 4.45-4.6 (1H, m), 4.65 (0.5H, d, J=8.5 Hz), 4.72 (0.5H, d, J=8.5 Hz), 5.22 (1H, s), 5.45 (0.5H, s), 5.54 (0.5H, s), 6.13 (1H, s), 6.8-6.9 (2H, m), 7.0-7.1 (1H, m), 7.15-7.40 (8H, m), 7.90 (0.5H, s), 8.16 (1H, m), 8.85 (1H, m), 9.34 (0.5H, s), 9.36 (0.5H, s).

Example 36

(3S, 4SR)-3-[2S-2-(6-N,N'-dibenzyloxycarbonylguanidino hexanoyl) amino-2-benzyl-acetamido]-4-phenylthio-azetidin-2-one

To a solution of thiophenol (110 mg, 1 mmole) in THF (5 ml) and 1N NaOH (1 ml, 1 mmole), (3S, 4S)-3-[2S-2-(6-N,N'-dibenzyloxycarbonylguanidino hexanoyl) amino-2-benzyl-acetamido]-4-acetoxy-azetidin-2-one (500 mg, 0.7 mmole) in THF (10 ml) and H_2O (1 ml) is added at 0 °C. The mixture is stirred at 0 °C for 1 hr and then at room temperature for 2 hr. After removal of solvent, the residue is dissolved in ethyl acetate, washed with water, brine and dried over sodium sulfate. After removal of solvent, the residue is purified by silica gel column chromatography using hexane-ethyl acetate as eluent. 270 mg of the title compound is obtained.

Yield: 50 %.

^{1}H-NMR (DMSO-d6), δ (ppm): 1.0-1.5 (6H, m), 2.05 (2H, m), 2.7-2.85 (1H, m), 3.0-3.15 (1H, m), 3.2-3.35 (2H, m), 4.45-4.7 (1H, m), 4.53 (0.6H, dd, J=8.3 & 2.1 Hz), 4.92 (0.6H, d, J=2.1 Hz), 5.03 (2H, s), 5.20 (2H, s), 5.26 (0.4H, d, J=4.5 Hz), 5.35-

5.45 (0.4H, m), 7.15-7.5 (20H, m), 8.07 (0.6H, d, J=8.3 Hz), 8.03 (0.4H, d, J=8.3 Hz), 8.37 (1H, m), 8.79 (0.6H, d, J=8.5 Hz), 9.00 (0.4H, d, J=8.5 Hz), 9.02 (0.6H, s), 9.06 (0.4H, s), 11.59 (1H, s).

Example 37

(3S, 4SR)-3-[2S-2-(6-N,N'-dibenzyloxycarbonylguanidino hexanoyl) amino-2-benzyl-acetamido]-4-phenylsulfonyl-azetidin-2-one

A mixture of (3S, 4SR)-3-[2S-2-(6-N,N'-dibenzyloxycarbonylguanidino hexanoyl) amino-2-benzyl-acetamido]-4-phenylthio-azetidin-2-one (220 mg, 0.29 mmole) and $KMnO_4$ (62 mg, 0.39 mmole) in acetic acid (5 ml) and water (1 ml) is stirred at 5 °C for 1 hr and then at room temperature for 1 hr. One drop of H_2O_2 (30% aq) is added. The reaction mixture is partitioned between ethyl acetate and water, the organic layer is washed with water, saturated NaHCO3, water, brine and dried over sodium sulfate. After removal of the solvent, solid is washed with ether and 220 mg of the title compound is obtained.

Yield: 95 %.

^{1}H-NMR (DMSO-d6), δ (ppm): 0.9-1.5 (6H, m), 1.99 (2H, m), 2.6-2.8 (1H, m), 2.9-3.1 (1H, m), 3.15-3.3 (2H, m), 4.4-4.7 (1H, m), 4.85-5.0 (1.2H, m), 5.01 (2H, s), 5.18 (2H, s), 5.20 (0.4H, m), 5.5-5.6 (0.4H, m), 7.1-7.5 (15H, m), 7.6-8.0 (5H, m), 8.03 (0.4H, d, J=8.3 Hz), 8.07 (0.6H, d, J=8.3 Hz), 8.34 (1H, m), 8.77 (0.6H, d, J=8.5 Hz), 8.73 (0.4H, d, J=8.5 Hz), 9.31 (0.6H, s), 9.43 (0.4H, s), 11.57 (1H, s).

Example 38

(3S,4R)-3-{2(S)-2-[6-(biotinoylamino)hexanoyl]amino-2-benzyl-acetamido}-4-phenoxy - azetidin-2-one

A suspension of 6-(biotinoyl)aminohexanoic acid (250 mg., 0.7 mmol) in dry DMF was heated to 60 °C for 10 min. and was added DCC (144 mg., 0.7 mmol), then N-hydroxy succinimide(100 mg., 0.875 mmol). After stirring for 6h. at room temp. the suspension obtained was filtered and treated with a solution of (3S, 4R)-3-(2S-2-amino-

2-benzyl-acetamido)-4-phenoxy-azetidin-2-one (321 mg, 0.7 mmol) and stirred at r.t. for 6h. The reaction mixture was concentrated in vacuo to give a solid which was triturated with ether then stirred with aq.sat. NaHCO3 solution(20 ml), filtered, washed with water, methanol followed by acetonitrile and air dried to give 102 mg of title compound as a solid.

Yield: 22%

^{1}H-NMR (DMSO-d6), δ (ppm): 1.00-1.70(m, 11, aliph-H), 1.92-2.10(m, 3H, btn-H), 2.48-3.19(m, 5H, aliph and CH2Ph), 4.10-4.15(m, 1H, btn-H), 4.30-4.66(m, 6H, CHCH2Ph, btn and aliph-H), 5.32-5.40(dd, 1h, J=7.5 and 3.5 Hz, C3H), 5.74(d, 1H, J= 3.8 Hz, C4H), 6.15-6.65(br s, 2H, NHCONH), 6.94-7.35(m, 10H, ar), 7.73(t, 1H, J=5.0 Hz, NH), 7.96(d, 1H, J= 8.8Hz , NH), 8.87(d, 1H, J=9.2 Hz, NH), 9.33(s,1H, ring NH).

Example 39

(3S,4S)-3-{2(S)-[5-[4-(pyrimidine-2-yl)piperazine]-5-oxopentanoyl]amino-2-benzyl acetamido}-4-phenoxy-azetidin-2-one

A solution of (3S,4S)-3-(2S-2-amino-2-benzyl-acetamido)-4-phenoxy-azetidin-2-one(147 mg, 0.452 mmol) in dry DMF (10ml) under nitrogen was treated with glutaric anhydride and stirred for 2hrs. at room temp.. The reaction mixture was treated with DCC (93.3 mg, 0.452 mmol) followed by 1-HOBT(61.1 mg, 0.452 mmol), stirred for 3hrs., then was added 2-pyrimidyl-1-piperazine dihydrochloride(107.2 mg, 0.452 mmol) followed by TEA. After stirring for 4 hrs., DMF was removed *in vacuo* to give a gum, which was dissolved in EtOAc, washed with aq.sat. NaHCO3 solution, brine, dried over Na2SO4, filtered and evaporated in vacuo to obtain a foam. Purification of the above crude product by prep.tlc (EtOAc:MeOH/ 8.5:1.5) gave 109 mg of title compound as a white solid.

Yield:. 41.2%; m.p.: 191-193 °C

^{1}H-NMR (DMSO-d6), δ (ppm): 1.52-1.16(m, 2H, COCH2), 2.00-2.20(m, 4H, CH2CH2-CO), 2.58-2.96(m, 2H, CH2Ph), 3.49-3.78(m, 8H, pip-H), 4.55-4.68(m, 1H, CHCH2Ph), 5.35(dd, 1H, J=5.0Hz and 2.5 Hz, C3H), 5.74(d, 1H, J=3.8 Hz, C4H), 6.67(t, 1H, J=

4.8Hz ,prm-H), 6.90-7.33(m, 10H, ar), 8.05(d, 1H, J= 8.5 Hz, CONH), 8.40(d, 2H, J= 4.5 Hz, prm-H), 8.90(d, 1H, J=9.1Hz, CONH), 9.3(s, 1H, ring-NH)

Example 40

(3S,4S)-3-{2(S)-[5-[4-(pyridin-2-yl)piperazine]-5-oxopentanoyl]amino-2-benzyl-acetamido}-4-phenoxy-azetidin-2-one

A solution of (3S, 4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)amino-4-phenoxy-azetidin-2-one (254 mg, 0.553 mmol) in a mixture of EtOAc:MeOH (1:1) was hydrogenated in presence of Pd-C(10%), at 50 psi over 6 hrs. and filtered through Celite. The solution was evaporated in vacuo to remove the volatiles and the solid obtained was

taken in DMF(18 ml).n the DMF solution was treated with glutaric anhydride(63 mg, 0.553 mmol.), stirred for 3 hrs. The mixture was treated with DCC(114 mg, 0.553 mmol) followed by 1-HOBT(75 mg, 0.553 mmol), stirred for 4 hrs. and was treated with 1-(2-pyridyl)piperazine (90.3 mg, 0.553 mmol) in DMF(1 ml). The reaction mixture was stirred over night at r.t., filtered and DMF was removed in vacuo. The crude product was taken in EtOAc, washed with aq. Sat. NaHCO3, brine, dried over Na2SO4, filtered and evaporated in vacuo to give a foam. The above foam was purified over prep. TIC plates using a mixture of EtOAc:MeOH(7:3) to give 130 mg of title compound.
Yield: 40.3%
^{1}H-NMR (DMSO-d6), δ (ppm): 1.67(t, 2H, J=7.0 Hz, COCH2), 2.10-2.26 (m, 4H, -CH2CH2CO), 2.73-3.15(m, 2H, CH2Ph), 3.40-3.53(m, 4H, pip-H), 4.45-4.60(m, 1H, CHCH2Ph), 4.66(d, 1H, J= 7.9Hz, C3H), 5.53(s, 1H, C4H), 6.66-7.62(m, 13H, ar), 8.14 (dd, 1H, J= 3.0Hz and 1.5 Hz, py-H), 8.21(d, 1H, J= 9.0 Hz, NH), 8.89(d, 1H, J= 8.4 Hz, NH), 9.32(s, 1H, ring NH).

Example 41

(3S,4S)-3-[2S-2-[4-(4-quinazolinyl)oxy-butanoyl]amino-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one

A solution of (3S, 4R)-3-(N-benzyloxycarbonyl-L-phenylalanyl)amino-4-phenoxy-

azetidin-2-one(218 mg, 0.461 mmol) in a mixture of THF:EtOAc:MeOH(2:2:1, 30 ml) was hydrogenated at 50 psi over 6 hrs in presence of 10% Pd-C, and filtered through Celite in to a suspension of benzotriazolyl 4-(4-quinazolinyl)oxybutyrate, prepared by reacting 4-(4-quinazolinyl)oxybutyric acid(107 mg, 0.461 mmol) with DCC (95 mg, 0.461 mmol) and 1-HOBT(63 mg, 0.461 mmol) in dry DMF(5 ml) for 1 h.. The suspension was allowed to stir at r.t. for 2 hrs. and filtered. The filtrate was evaporated in vacuo, and the crude mass was dissolved in EtOAc, washed with aq. Sat. NaHCO3, brine, dried over Na2SO4 and evaporated in vacuo to give an off white solid. Purification of the above product by silica gel column chromatography (gradient mix. of Hexanes:EtOAc, 1:1 to 1:4) gave 50 mg desired product.

Yield: 29%

^{1}H-NMR (DMSO-d6), δ (ppm): 1.80-1.90(m, 2H, aliph-H), 2.05-2.15(m, 2H, aliph-H), 2.70-3.10(m, 2H, CH2Ph), 3.80-4.05(m, 2H, aliph-H), 4.44-4.55(m, 1H, CHCH2Ph), 4.64(d, 1H, J=8.7 Hz, C3H), 5.52(s, 1H, C4H), 6.80-7.90(m, 13H, ar), 8.16(d, 1H, J= 8.0 Hz, qn-H), 8.28(s, 1H, qn-H), 8.34(d, 1H, J= 6.2 Hz, NH), 8.88(d, 1H, J= 8.4 Hz, NH), 9.31(s, 1H, ring NH).

Example 42

(3S,4S)-3-[2S-2-[4-(3,5-dimethoxyphenyl)oxybutanoyl]amino-2-benzyl-acetamido]-4-phenoxy-azetidin-2-one

A solution of (3S,4R)-3-(N-benzyloxycarbonyl-L-phenylalanyl)amino-4-phenoxy-azetidin-2-one(147 mg, 0.32 mmol) in a mixture of THF:EtOAc (1:1, 13 ml) was hydrogenated at 50 psi over 4 hrs in presence of 10% Pd-C, and filtered through Celite in to a suspension of benzotriazolyl 4-(3,4-dimethoxyphenyl)oxybutyrate, prepared by reacting 4-(3,4-dimethoxyphenyl)oxybutyric acid(133 mg, 0.32 mmol) with DCC (66 mg, 0.32 mmol) and 1-HOBT(43 mg, 0.32 mmol) in dry DMF(5 ml) for 1 hr.. The suspension was allowed to stir at r.t. for 6 hrs. and filtered. The filtrate was evaporated in vacuo, and the crude mass was dissolved in EtOAc, washed with aq. Sat. NaHCO3, brine, dried over Na2SO4 and evaporated in vacuo to give an off white solid.

Purification of the above product by silica gel chromatography using a mixture of Hexanes:EtOAc (1:1) gave 50 mg. of title compound.

Yield: 29 %

^{1}H-NMR (DMSO-d6), δ (ppm): 1.75-1.85(m, 2H, aliph-H), 2.20-2.32(m, 2H, aliph-H), 2.70-3.11(m, 2H, CH2Ph), 3.70(s, 6H, 2-OCH3), 3.75-4.06(m, 2H, aliph-H), 4.49-4.59(m, 1H, CHCH2Ph), 4.65(d, 1H, J= 7.8 Hz, C3H), 5.52(s, 1H, C4H), 6.05-6.08(m, 2H, aromatic protons), 6.80-7.30(m, 11H, aromatic protons), 8.25(d, 1H, J= 8.5 Hz, NH), 8.89(d, 1H, J= 8.3 Hz, NH), 9.33 (s, 1H).

Example 43

(3S,4S)-3-{2-2S-[2-(E)-(2-Thienyl)-2-[2-(t-butoxycarbonylamino)ethoxyimino) acetamido] -2-cyclohexylmethyl-acetamide}amino-4-phenoxy-azetidin-2-one

A solution of (E)-2-(2-Thienyl)-2-[1-(t-butoxycarbonylamino)ethoxyimino)] acetic acid(143 mg, 0.455 mmol) in dry DMF(5 ml) was treated with DCC(94 mg, 0.455 mmol) followed by HOBT(62 mg, 0.455 mmol) and stirred for 1.5 hrs. The resulting suspension was filtered and was added a solution of (3S, 4S)-3-[2-amino-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one (151 mg, 0.455 mmol) in DMF(2 ml). After stirring for 2 hrs. DMF was removed *in vacuo* and the crude product obtained was triturated with 20% i-propanol in ether, filtered and air dried to give 274 mg of the title compound.

Yield: 96%; m.p.: 77-79 ^{0}C

^{1}H-NMR (DMSO-d6), δ (ppm): 0.85-1.80(m, 22H, Bu^t, CH_2 and Chx proptons), 3.30(m,2H, NCH_2), 4.24-4.30(m, 2H, $-OCH_2$),, 4.43-4.55(m, 1H, C<u>H</u>CH_2Chx), 4.74(d, 1H, $J_{= 8.6\ Hz}$, C_3H), 5.55(s, 1H, C_4H), 6.88-7.88(m, 9H, ar and –BocNH), 8.65(d, 1H, $J_{= 7.6\ Hz}$, NH), 9.04(d, 1H, $J_{= 8.0Hz}$, NH), 9.33(s, 1H, ring NH)

Example 44

(3S,4S)-3-{2-2S-[2-(E)-(2-Thienyl)-2-[2-aminoethoxyimino)acetamido]-2-cyclohexylmethyl-acetamido}-4-phenoxy-azetidin-2-one

A solution of (3S,4S)-3-{2-2S-[2-(E)-(2-Thienyl)-2-[2-(t-butoxycarbonylamino) ethoxyimino) acetamido] -2-cyclohexylmethyl-acetamide}amino-4-phenoxy-azetidin-2-one (190 mg, 0.304 mmol) in dry DCM (8 ml) under nitrogen was cooled to 0° C and was added TFA(2 ml). After stirring for 1.5 hrs. the volatiles were evaporated *in vacuo* and the gum obtained was dissolved in EtOAc. The EtOAc solution was washed with aq. Sat. $NaHCO_3$, brine, dried over $MgSO_4$ and evaporated in vacuo to give a solid. The above sample was triturated with ether, filtered and air dried to give 66 mg of title compound.

Yield: 40%; m.p.: 91-93 °C

^{1}H-NMR (DMSO-d6), δ (ppm): 0.90-1.77(m, 13H, CH_2Chx, Chx), 2.88-2.99(m, 4H, NCH_2CH_2O), 3.00-3.52(br s, 2H, NH_2), 4.50-4.54(m, 1H, C<u>H</u>CH_2Chx), 4.74(d, 1H, J= 8.6 Hz, C_3H), 5.55(s, 1H, C_4H), 6.91-7.88(m, 8H, ar.), 8.72(d, 1H, J= 7.6 Hz, NH), 8.99(d, 1H, J= 8.5 Hz, NH), 9.32(s, 1H, ring NH)

<u>Example 45</u>

<u>(3S,4S)-3-{2S-2-[4-(4-amidinophenyl)aminocarbonyl-butanoyl]amino-2-benyzl-acetamido} -4-acetoxy-azetidin-2-one :</u>

4-Aminobenzamidine dihydrochloride (520 mg, 2.5 mmol) was added to dry DMF (3ml). To this solution were added pyridine (2 ml) and glutaric anhydride (285 mg, 2.5 mmol) followed by dimethylamino pyridine (31 mg, 0.25 mmol). The product precipitated after heating for 1 hr. at 100 °C. The reaction mixture was diluted with 5 ml of water, filtered, washed with water(10ml), acetonitrile(10 ml) and ether (30 ml). The solid was suspended in dioxane and was added 4 N HCl to adjust the pH to ~2 and freeze dried to give 4-[(4-amidinophenyl)aminocarbonyl-butanoic acid as a white solid (268 mg, 38%). The above acid (250 mg, 0.88 mmol) was disolved in dry DMF (10 ml) and treated with N-methyl morpholine(89 mg, 0.88 mmol) followed by isobutyl chloroformate (120 mg, 0.88 mmol) at 25 °C. The mixture was srirred for 5 min and was added a solution of (3S, 4S)-3-(2S-2-amino-2-benzyl-acetamido)-4-acetoxy-azetidin-2-one in THF(16ml) prepared from the (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-

benzyl-acetamido)-4-acetoxy-azetidin-2-one (372 mg, 0.88 mmol), by hydrogenation at 50 psi over 4 hrs. in presence of 10% Pd-C (372 mg, 50% wet). After 1 hr. the solvents were removed in vacuo and the residue was dissolved in acetonitrile/water and lyophilized. Purification of the crude product by repeated reverse phase column chromatography over HP-20 (water:acetonitrile/10:0 to 8:2) followed by reverse phase silica gel preparative TLC (Analtech@ RPSF plates), using H_2O:CH_3CN:AcOH(8.0:1.8:0.2) as developing solvent and lyophilization gave 180 mg of title compound.

Yield: 37%

^{1}H-NMR (DMSO-d6), δ (ppm): 1.24-2.35(m, 9H, $COCH_3$ and $CO(CH_2)_3CO$), 2.61-3.20(m, 2H, CH_2Ph), 4.46-4.67(m, 2H, C_3H and $CHC\underline{H}_2Ph$), 5.75(s, 1H, C_4H), 7.24(s, 5H, ar), 7.71(s, 4H, ar.), 6.50-7.90(br, 4H, amidine-H), 8.19(br s, 1H, NH), 8.75(br s, 1H, NH), 9.22(br s, 1H, ring NH), 10.20(br s , 1H, NHPh)

Example 46

(3S, 4S)-3-{2S-2-[5-(N, N-dibenzyloxycarbonylguanidino)-(2S)-2-benzyloxycarbonyl amino-pentanoylamino]-2-benzyl-acetamido}-4-acetoxy- azetidine-2-one

By a similar method as described in example 2, the title compound is obtained from 5-(N,N-dibenzyloxycarbonyl-guanidino)-(2S)-2-benzyloxycarbonylamino-pentanoic acid and (3S, 4S)-3-[2S-2-amino-2-benzyl-acetamido]-4-acetoxy-azetidin-2-one.

Yield: 44 %

^{1}H NMR (DMSO-d_6): 1.50-1.76(m, 3H), 2.06(s, 3H), 2.76-3.05(m, 2H), 3.75(br s, 2H), 3.90(br s, 1H), 4.44-4.54(m, 1H), 4.63(d, 1H, J=8.1Hz), 5.00(s, 2H), 5.04(s, 2H), 5.21(s, 2H), 5.69(s, 1H), 7.17-7.37(m, 20H), 7.47(d, 1H, J=7.8Hz), 7.97(d, 1H, J=8.1Hz), 8.73(d, 1H, J=8.2Hz), 9.15(br s, 2H), 9.24(s, 1H).

Example 47

(3S, 4S)-3-[2S-2-(6-guanidinohexanoyl)-amino-2-benzyl-acetamido]-4-acetoxy- azetidin-2-one hydrochloride salt

By a similar method as described in example 2, the title compound is obtained from 6-(N,N=-dibenzyloxycarbonyl-guanidino)-hexanoic acid and (3S, 4S)-3-[2S-2-amino-2-benzyl-acetamido]-4-acetoxy-azetidin-2-one.

Yield: 74%

^{1}H NMR (DMSO-d_6): 1.0-1.6 (6H, m), 2.0-2.2 (5H, m), 2.7-3.2 (4H, m), 4.5-4.7 (1H, m), 4.59 (1H, d, J=8.4 Hz), 5.76 (1H, s), 6.9-7.8 (10 H, m), 8.14 (1H, d, J=8.4 Hz), 8.84 (1H, d, J=8.4 Hz), 9.22 (1H, s)

Example 48

(3S, 4S)-3-[2S-2-(6-guanidinohexanoyl)-amino-2-benzyl-acetamido]-4-phenylthio-azetidin-2-one trifluoroacetic acid salt

By a similar method as described in example 2, (3S, 4S)-3-{2S-2-[6-(N,N=-di-(t-butyloxycarbonyl) guanidino-hexanoyl] amino-2-benzyl-acetamido]-4-phenylthio-azetidin-2-one is obtained from 6-[N,N=-di-(t-butyloxycarbonyl) guanidino]-hexanoic acid and (3S, 4S)-3-[2S-2-amino-2-benzyl-acetamido]-4-phenylthio-azetidin-2-one.

To a solution of (3S, 4S)-3-{2S-2-[6-(N,N=-di-(t-butyloxycarbonyl) guanidino-hexanoyl] amino-2-benzyl-acetamido]-4-phenylthio- azetidin-2-one (90 mg, 0.13 mmole) and anisole (2 drops) in dichloromethane (2 ml), trifluoroacetic acid (0.7 ml) is added at 0 oC. The reaction mixture is stirred at 0 oC for 30 min and room temperature for 1 hr. the solution is evaporated to dryness under vacumm and the residue triturated with ether and hexane. 75 mg of the title compound is obtained as solid.

Yield: 95 %

^{1}H NMR (DMSO-d_6): 1.0-1.6 (6H, m), 2.0-2.2 (2H, m), 2.7-3.2 (4H, m), 4.5-4.7 (1H, m), 4.51 (1H, d, J=8.4 Hz), 4.93 (1H, s), 7.0-7.6 (15 H, m), 8.15 (1H, d, J=8.4 Hz), 8.85 (1H, d, J=8.4 Hz), 9.05 (1H, s).

Example 49

(3S, 4S)-3-[2S-2-(6-guanidinohexanoyl)-amino-2-benzyl-acetamido]-4-phenylsulfonyl-azetidin-2-one trifluoroacetic acid salt

A mixture of (3S, 4S)-3-{2S-2-[6-(N,N=-di-(t-butyloxycarbonyl) guanidino-hexanoyl] amino-2-benzyl-acetamido]-4-phenylthio- azetidin-2-one (350 mg, 0.5 mmole) in acetic acid (9.6 ml) and water (1.9 ml), and $KMnO_4$ (120 mg) is stirred at 0 °C for 30 min and then room temperature for 1 hr. few drops of H_2O_2 (30%) is added. The reaction mixture is partitioned between ethyl acetate and water, the organic layer is washed with water, saturated NaHCO3, water, brine and dried over Na_2SO_4. After removal of the solvent, solid is washed with ether and 200 mg of (3S, 4S)-3-{2S-2-[6-(N,N=-di-(t-butyloxycarbonyl) guanidino-hexanoyl] amino-2-benzyl-acetamido]-4-phenylsulfonyl-azetidin-2-one is obtained.

To a solution of (3S, 4S)-3-{2S-2-[6-(N,N=-di-(t-butyloxycarbonyl) guanidino-hexanoyl] amino-2-benzyl-acetamido]-4-phenylsulfonyl- azetidin-2-one (100 mg, 0.14 mmole) and anisole (2 drops) in dichloromethane (2 ml), trifluoroacetic acid (0.7 ml) is added at 0°C. The reaction mixture is stirred at 0°C for 30 min and room temperature for 1 hr. The solution is evaporated to dryness under vacumm and the residue triturated with ether. The resulting solid is dissolved in water and washed with ethyl acetate. The title compound (40 mg) is obtained as solid after removal of water by lyophilization.

Yield: 44 %

^{1}H NMR (DMSO-d_6): 1.0-1.6 (6H, m), 2.0-2.2 (2H, m), 2.6-2.7 (1H, m), 2.9-3.2 (3H, m), 4.4-4.6 (1H, m), 4.90 (1H, d, J=8.4 Hz), 4.98 (1H, s), 6.8-7.6 (10 H, m), 7.7-8.0 (5H, m), 8.12 (1H, d, J=8.4 Hz), 8.90 (1H, d, J=8.4 Hz), 9.35 (1H, s).

Example 50

(3S, 4R)-3-[2S-2-[4-(pyrimid-2-yl)-piperazino]acetamido-2-cyclohexylmethyl-acetamido]-4-phenoxy- azetidin-2-one

By a similar method as described in example 2, the title compound is obtained from 2-[4-(pyrimid-2-yl)-piperazino]acetic acid and (3S, 4R)-3-[2S-2-amino-2-cyclohexylmethyl-acetamido]-4-phenoxy-azetidin-2-one.

Yield: 45%

^{1}H NMR (DMSO-d_6): 0.7-1.7 (13H, m), 2.3-2.6 (4H, m), 2.95 (2H, s), 3.7-3.85 (4H, m), 4.35-4.45 (1H, m), 5.3-5.4 (1H, m), 5.75 (1H, d, J=3.8 Hz), 6.65 (1H, m), 6.8-7.0 (3H, m), 7.25-7.35 (2H, m), 7.74 (1H, d, J=8 Hz), 8.36 (2H, d, J=5 Hz), 8.77 (1H, d, J=8 Hz), 9.26 (1H, s).

Biological Example

Testing of inhibitors for inhibition of Cathepsin B, L, K and S

In vitro assay procedure for cathepsin B

The compounds of formula I were tested for inhibition of cathepsin B using the known method (A.J. Barret et al., Biochem. J. 1982, 201, 189-198). To a 170 µl of enzyme-buffer mixture (enzyme: r rat cathepsin B, diluted to give approximate 10 F units/min, buffer: 56 mM sodium acetate, 1.124 mM EDTA, 10 mM DTT, pH 5.1) a 10 µL of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, a 20 µl of 5 mM substrate (N-CBZ-Phe-Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10 min at the fluoroscan reader

(excitation at 380 nm emission at 460 nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC_{50} is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

In vitro assay procedure for cathepsin L

To a 170 µl of enzyme-buffer mixture (enzyme: r rat cathepsin L) diluted to give approximate 15 F units/min, buffer: 58.8 mM sodium citrate, 1.18 mM EDTA, 235 mM sodium chloride, 5 mM DTT, pH 5.0) a 10 µL of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, a 20 µl of 1 mM substrate (N-CBZ-Phe-Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10 min at the fluoroscan reader (excitation at 380 nm emission at 460 nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC_{50} is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

In vitro assay procedure for Cathepsin K

To a 170µl of enzyme-buffer mixture (enzyme r Cathepsin K) diluted to give appr 30 F units/min, buffer: 100mM sodium acetate, 5 mM EDTA, 20 mM L-Cysteine, 0.01% Brij , pH 5.5) a 10µl of inhibitor (dissolved in 100 % DMSO) was added.

After 10 min of incubation at room temperature a 20µl of 2.7 mM substrate (N-CBZ-Phe-Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10min at the Fluoroscan II plate reader (excitation at 380nm, emission at 460nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC_{50} is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

In vitro assay procedure for Cathepsin S

To a 170µl of enzyme-buffer mixture (enzyme: r Cathepsin S) diluted to give appr 30 F units/min, buffer: 100mM sodium phosphate, 1 mM EDTA, 5 mM DTT, 0.01% Brij, pH 6.5.) a 10µl of inhibitor (dissolved in 100 % DMSO) was added.

After 10 min of incubation at room temperature a 20µl of 1.2 mM substrate (CBZ-Val-Val-Arg- AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10min at the Fluoroscan II plate reader (excitation at 380nm, emission at 460nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC_{50} is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

Table 1. In vitro inhibitory activity of monobactam compounds on cysteine proteases

Example No.	IC_{50} (µM)			
	Cathepsin B	Cathepsin L	Cathepsin K	Cathepsin S
1	>50	0.081	2.5	>2.5
2	37	0.37	50	0.47
3	48	0.38	50	0.393
4	9.04	0.014	1.14	0.201
5	8.81	0.0028	27.7	0.073
6	48	1.91	>2.5	0.0329
7	0.57	0.08	0.1000	0.017
8	>50	2.26	>2.5	>2.5
9	>50	2.0	>2.5	>2.5
10	46	4.26	>2.5	0.303

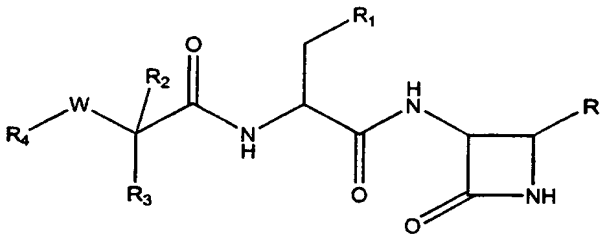
>50	2.6	>2.5	>2.5
49.8	2.0	>2.5	>2.5
0.075	1.1	1.9	0.053
4.3	0.39	0.88	0.021
1.94	0.08	0.5	0.1
0.91	0.32	>2.5	0.405
1.23	0.38	>2.5	1.077
8.2	4.8	>2.5	2.5
6	5.7	>2.5	0.225
9.1	9.1	>2.5	0.34
8.2	1.6	>2.5	2.5
>48	9.7	>2.5	0.32
9.1	0.36	1.41	1.086
>50	1.25	>2.5	>2.5
47	9.4	>0.1	2.5
>50	0.08	0.1000	0.19
43	1.7	1.9	0.87
5.8	0.42	>2.5	n.a
0.4	0.07	n.a	n.a
7.52	0.3	2.5	>2.5
23	0.31	2.5	0.41
0.026	0.6	0.1000	0.02
2.12	0.23	1.7	0.023
8.98	0.27	1.29	0.36
45.5	1.98	2.5	>2.5
28.5	5.36	2.5	2.5
28.1	0.17	0.0586	0.0069
0.30	0.18	0.1000	0.16
1.28	0.17	0.1000	2.5

40	43.0	4.28	2.5	>2.5
41	>50	3.33	1.94	>2.5
42	36.5	4.38	2.5	>2.5
43	7.9	0.32	>2.5	0.15
44	5.0	0.76	2.5	0.34
45	5.19	0.36	1.54	0.047
46	11.76	18.3	>2.5	0.0084
47	3.47	0.062	0.87	0.011
48	8.19	0.28	0.1000	0.36
49	19.37	0.062	0.0250	0.07
50	1.56	0.3	0.1	0.01

Claims.

1. A 3-(2-disubstituted acetamido)monobactam compound of formula (I), or a pharmaceutically acceptable salt thereof:

(I)



wherein

W is a divalent cycloalkyl radical, a bond, or a divalent $-(CH_2)_n-$ radical wherein n is an integer from 1 to 8 inclusive;

R is a group $-OCOR_5$ or a group $-X_1R_7$ wherein

R_5 is

(i) a C_1-C_6 alkyl group which may be substituted by 1 or 2 substitutents selected from

hydroxy,
halogen,
amino,
carboxy
guanidino,
amidino,
phenyl, or
phenyl substituted by 1 to 3 substituents selected from

hydroxy,
methylendioxy,
halogen,
carboxy
C_1-C_4 alkyl,
C_1-C_4 alkoxy,
cyano,
guanidino,
amidino,
amino or
-NHCOR$_6$ wherein R$_6$ is C_1-C_4 alkyl; or

(ii) an aryl group which may be substituted by 1 to 3 substituents selected from

hydroxy,
methylendioxy,
halogen,
carboxy,
C_1-C_4 alkyl,
C_1-C_4 alkoxy,
cyano,
guanidino,
amidino, or
NHCOR$_6$ wherein R$_6$ is C_1-C_4 alkyl; or

X_1 is -O-, -S-, -S(O)-, or -S(O_2)-;

R_7 is

(i) a C_1-C_6 alkyl group which may be substituted by 1 to 2 substitutents

selected from

hydroxy,
carboxy,
halogen,
amino,
phenyl, or
phenyl substituted by 1 to 3 substituents selected from
hydroxy,
methylendioxy,
halogen,
carboxy,
phenyl,
C_1-C_4 alkoxy,
cyano,
heterocyclyl,
C_1-C_4 alkyl, or
C_1-C_4 alkyl substituted with carboxy and/or amino;

(ii) a cycloalkyl group;

(iii) an aryl group which may be substituted by 1 to 3 substituents selected from

hydroxy,
methylendioxy,
amino,
cyano,
halogen,
carboxy,
phenyl,

C_1-C_4 alkoxy,
C_1-C_3-haloalkyl,
heterocyclyl,
C_1-C_4 alkoxy,
cyano.
heterocyclyl,
C_1-C_4 alkyl, or
C_1-C_4 alkyl substituted with carboxy and/or amino; or

(iv) a heterocyclic group, which may be substituted by 1 or 2 substituents, selected from
hydroxy,
halogen,
carboxy,
C_1-C_4 alkyl,
C_1-C_4 alkoxy or
cyano;

(a) an aryl group which may be substituted by 1 to 3 substitutents selected from C_1-C_4 alkyl, C_1-C_3-haloalkyl, halogen, -$NHCOR_8$, -OR_8, -NHR_8, -$N(R_8)_2$, -SR_8, phenyl, amidino, or guanidino, wherein R_8 is hydrogen, C_1-C_6 alkyl, C_3-C_6 cycloalkyl or phenyl; or

(b) a heterocyclic group, which may be substituted by 1 to 2 substitutents, selected from C_1-C_4 alkyl, C_1-C_4 alkoxy, hydroxy, halogen, carboxy, amino, cyano or phenyl; or

(c) cycloalkyl; or

(d) C_1-C_4 alkyl;

R_2 is hydrogen and R_3 is hydrogen or an amino or mono- or di-(C_1-C_6)alkylamino group, an acylamino group, or an aryloxycarbonyl- or (C_1-C_6)alkoxycarbonylamino group; or

R_2 and R_3 taken together with the carbon atom to which they are attached form a cycloalkyl ring; or

R_2 and R_3 taken together represent a group =NOR$_9$ wherein R_9 is (C_1-C_6)alkyl optionally substituted by an amino, aryloxycarbonyl- or (C_1-C_6)alkoxycarbonylamino group; and

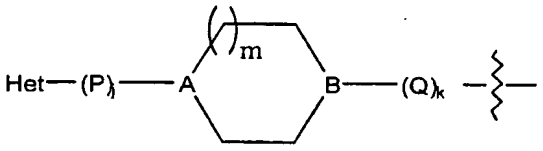
R_4 is (a) a group -NH-C(=NR$_{11}$)R$_{10}$ wherein R_{10} is amino, mono- or di-(C_1-C_6)alkylamino, protected amino, or (C_1-C_6)alkyl, and R_{11} is hydrogen, (C_1-C_6)alkyl, or an N-protecting group; or

(b) a (C_2-C_{12})alkyl-, cycloalkyl(C_2-C_{12})alkyl-, heterocyclyl(C_2-C_{12})alkyl-, aryl(C_2-C_{12})alkyl-, heteroaryl(C_2-C_{12})alkyl- group, the (C_2-C_{12})alkyl part of which which is interrupted by one or more non-adjacent O or S atoms; or

(c) a (C_1-C_6)alkoxy-, aryloxy-, cycloalkyl(C_1-C_6)alkoxy-, heterocyclyl(C_1-C_6)alkoxy-, aryl(C_1-C_6)alkoxy-, heteroaryl(C_1-C_6)alkoxy-, (C_1-C_6)alkylthio-, arylthio-, cycloalkyl((C_1-C_6)alkylthio-, heterocyclyl(C_1-C_6)alkylthio-, aryl(C_1-C_6)alkylthio-, or heteroaryl(C_1-C_6)alkylthio- group any of which may be substituted by hydroxy, methylenedioxy, (C_1-C_4)alkoxy, (C_1-C_4)alkyl, halogen, cyano, carboxy, amino, or a group -NH-C(=NH)R$_{12}$ wherein R_{12} is amino or (C_1-C_6)alkyl; or

(d) an aryl, cycloalkyl, or heterocyclic group, or an amino group substituted with an N-containing heterocyclic group; or

(e) a group of formula F



(F)

wherein P and Q are independently -C(=O)-, -S(O_2)- or -CH_2-, provided at least one of P and Q is not -CH_2-; j, k and m are independently 0 or 1, provided at least one of j and k is 1; A and B are independently nitrogen or carbon atoms, provided at least one of A and B is a nitrogen atom; and "Het" is a heterocyclic group having at least one ring nitrogen atom;

(f) a group -$NHCOCH_2OR_{13}$, -$NHCOCH_2SR_{13}$, -$NHCOCH_2SO_2R_{13}$, or -$COOR_{13}$ wherein R_{13} is (C_1-C_6)alkyl, aryl, heterocyclic or cycloalkyl, which may be substituted by 1-3 substituents selected from hydroxy, methylenedioxy, halogen, cyano, carboxy, guanidino, amidino, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, amino or phenyl.

2. A compound as claimed in claim 1 wherein W is a divalent -$(CH_2)_n$- radical wherein n is an integer from 1 to 6 inclusive.

3. A compound as claimed in claim 1 or claim 2 wherein R is a group -X_1R_7 wherein X_1 is -O-, and R_7 is an phenyl group which may be substituted by carboxy, C_1-C_4 alkyl, or C_1-C_4 alkyl substituted with carboxy and/or amino.

4. A compound as claimed in any of claims 1 to 3 wherein R_1 is C_1-C_4 alkyl, cyclohexyl, or a phenyl or naphthyl group, which phenyl or naphthyl group may be substituted by C_1-C_4 alkyl, C_1-C_3-haloalkyl, halogen, -$NHCOR_8$, -OR_8, -NHR_8, -$N(R_8)_2$, or -

SR_8, wherein R_8 is hydrogen or C_1-C_4 alkyl.

5. A compound as claimed in any of the preceding claims wherein R_2 and R_3 are each hydrogen.

6. A compound as claimed in any of claims 1 to 4 wherein R_2 is hydrogen and R_3 is an amino, acylamino, aryloxycarbonylamino or (C_1-C_6)alkoxycarbonyl-amino group.

7. A compound as claimed in claim 6 wherein R_2 is hydrogen and R_3 is an benzyloxycarbonylamino, tert-butoxycarbonylamino or acetylamino group.

8. A compound as claimed in any of claims 1 to 4 wherein R_2 and R_3 taken together with the carbon atom to which they are attached form a cyclopropyl or cyclohexyl ring.

9. A compound as claimed in any of claims 1 to 4 wherein R_2 and R_3 taken together represent a group $=NOR_9$ wherein R_9 is (C_1-C_4)alkyl group which is substituted by an amino, benzyloxycarbonylamino, tert-butoxycarbonylamino or acetylamino group.

10. A compound as claimed in any of the preceding claims wherein R_4 is a group $-NH-C(=NR_{11})R_{10}$ wherein R_{10} is amino, benzyloxycarbonylamino, tert-butoxycarbonylamino, acetylamino, or methyl, and R_{11} is hydrogen, benzyloxycarbonyl, tert-butoxycarbonyl, or acetyl.

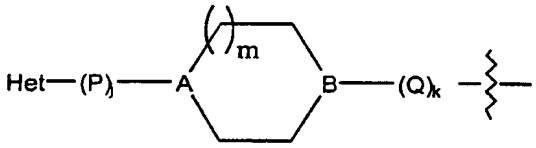
11. A compound as claimed in any of claims 1 to 9 wherein R_4 is a (C_2-C_6)alkyl-, (C_3- C_7)cycloalkyl(C_2-C_6)alkyl-, heterocyclyl(C_2-C_6)alkyl- wherein the heterocyclic ring is monocyclic with 5 or 6 ring atoms at least one of which is nitrogen, or phenyl(C_2-C_6)alkyl-group, the (C_2-C_6)alkyl part of any of the foregoing being interrupted by one or more non-adjacent O atoms.

12 A compound as claimed in claim 11 wherein R_4 is a 2-methoxyethoxy or 2(-2-methoxyethoxy)ethoxy group.

13. A compound as claimed in any of claims 1 to 9 wherein R_4 is a (C_1-C_6)alkoxy-, carboxymethoxy, carboxymethylthio, phenoxy, 3,4-methylendioxyphenoxy, 3,4-dimethoxyphenoxy, 2-, 3-, or 4-pyridinyloxy, 2-, 3-, or 4-pyridinylthio, benzimidazolyloxy, benztriazolyloxy, naphthyloxy, or benzyloxy group.

14 A compound as claimed in any of claims 1 to 9 wherein R_4 is a 2-, or 3-thienyl, phenyl, 2-, 3- or 4-pyridinyl, cyclohexyl, or triazolylamino group.

15 A compound as claimed in any of claims 1 to 9 wherein R_4 is a group of formula F



(F)

wherein m and k are each 1, j is 0, Q is -C(=O)-, A and B are both nitrogen atoms, and "Het" is a monocyclic heterocyclic group having 5 or 6 ring carbon atoms wherein the ring hetero atom(s) are nitrogen.

16. A compound as claimed in any of the preceding claims wherein the stereochemical configuration at the two asymmetric carbons 3 and 4 respectively of the azetidin-2-one ring system is predominantly (3R,4S), (3R,4R), (3S,4R) or (3S,4S).

17. A compound as claimed in any of the preceding claims, wherein the group R_1CH_2- is the side chain of a non-natural D- or L-amino acid.

18. A compound as claimed in any of the preceding claims which is in the form of a sodium, potassium, magnesium, calcium, hydrogen chloride, tartaric acid, succinic acid, fumaric acid or p-toluenesulfonic acid salt.

19. A pharmaceutical composition comprising af compound as claimed in any of the preceding claims together with a pharmaceutically acceptable carrier.

20. A method for the treatment of diseases susceptible to amelioration by inhibition of cysteine protease activity, comprising administration to the patient of an amount of a compound as claimed in any of the preceding claims effective to inhibit such activity.

21. A method as claimed in claim 20 wherein the disease is muscular dystrophy, bone resorption, arthritis, myocardial infarction, or cancer metastasis and/or growth.

INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/IB 99/00558

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K5/065 C07K5/087 A61K38/05 A61K38/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y Y	WO 96 32408 A (SYNPHAR LAB INC) 17 October 1996 cited in the application see claim 1 and examples	1-7,11, 14,16, 18-21 8-10,12, 13,15,17
Y	WO 97 38008 A (SYNPHAR LAB INC ;CANADA NAT RES COUNCIL (CA)) 16 October 1997 see page 5	1-21
P,X	WO 98 12210 A (SYNPHAR LAB INC ;CANADA NAT RES COUNCIL (CA)) 26 March 1998 see claims and examples	1-7, 10-21
P,X	WO 98 12176 A (SYNPHAR LAB INC) 26 March 1998 see claims and examples	1-7, 10-21

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 June 1999	05/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Steendijk, M

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INTERNATIONAL SEARCH REPORT

In' itional Application No
PCT/IB 99/00558

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 32766 A (SYNPHAR LAB INC) 30 July 1998 see page 16, scheme I, compound 2	1,2,4-7, 10-17

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int tional Application No
PCT/IB 99/00558

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		CA 2212356 A	17-10-1996
		EP 0817795 A	14-01-1998
		JP 11503728 T	30-03-1999
WO 9738008 A	16-10-1997	AU 2174497 A	29-10-1997
		CA 2252086 A	16-10-1997
		EP 0904284 A	31-03-1999
WO 9812210 A	26-03-1998	AU 4133597 A	14-04-1998
WO 9812176 A	26-03-1998	AU 4133697 A	14-04-1998
WO 9832766 A	30-07-1998	AU 5675998 A	18-08-1998